Analysis of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) diversity towards improved yield

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DECLARATION AND APPROVAL

Declaration by candidate

I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agricultural Sciences, School of Environmental and Health Sciences, and the work contained herein is my original work and that it has not been submitted at any other University in part or entirely for the award of any degree.

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DEDICATION

This work is dedicated to the Jehovah Shalom and my family.

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Table A. Contribution of all authors and consent of use of manuscripts as part of this thesis

Author	Article	Contribution	Assent
O.S. Olanrewaju	Article 1-5	Principal investigator: Responsible for conducting the study design, managing literature searches, conducting the study, data collection, analysis, reporting, and interpretation. Also, the first author responsible for writing of articles and thesis.	Olanrewaju Oluwaseyi Digitally signed by Olanrewaju Oluwaseyi Date: 2021.10.29 07:42:02 +02'00'
O. Oyatomi	Article 1-5	Supervised the study design, provided intellectual input on the study design, also gave guidance on data collection and writing of articles and thesis.	Dr. Oyatomi Olaniyi Ajewole. Digitally signed by Dr.Oyatomi Olaniyi Ajewole Date:2021:11:19 07:11:00 +01
O.O. Babalola	Article 1-5	Promoter: Supervised the study design and monitored the progress. Also provided intellectual input during the writing of articles and thesis.	Prof Digitally signed by Prof Oluranti BABALOLA Date: 2021.10.29 06:13:07 +02'00'
M. Abberton	Article 1-5	Co-promoter: Supervised the study design and monitored the progress. Also provided intellectual input during the writing of articles and thesis.	Signed by Prof. Michael Abberton date: 22nd Nov. 2021

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LIST OF ABBREVIATIONS

AD	Agricultural Drought
AFLP	Amplified Fragment Length Polymorphism
AMMI	Addictive Main Effects Multiplicative Interaction
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BGN	Bambara Groundnut
BNF	Biological Nitrogen Fixation
Ca	Calcium
CGIAR	Consortium of International Agricultural Research
CHLCON	Chlorophyll Content
CMLM	Compressed mixed linear model
DArT	Diversity Array Technology
DNA	Deoxyribonucleic Acid
DT50G	Days to 50% Germination
DTE	Days to Emergence
DTF	Days to First Flowering
ECEC	Effective Cation Exchange Capacity
FAO	Food and Agriculture Organization
F-LSD	Fischer's Least Significant Difference
GBS	Genotyping by Sequencing
GCT	Germination Count
GEI	Genotype Environment Interaction
GGE	Genotypic Main Effect Plus Genotype-By-Environment Interaction

GLM	Generalized Linear Model
GWAS	Genome-Wide Association Studies
HSWT	Hundred Seed Weight
IITA	International Institute of Tropical Agriculture
K	Potassium
LLE	Leaf Length
LS	Leaf Senescence
LSD	Least Significant Difference
LWI	Leaf Width
MAS	Marker-Assisted Selections
MET	Multi-Environment Trials
Mg	Magnesium
N	Nitrogen
NGS	Next-Generation Sequencing
NPET	Number of Petioles
NPOD	Number of Pods
NSEED	Number of Seeds
OC	Organic Carbon
P	Phosphorus
PCA	Principal Component Analysis
PEL	Petiole Length
PH	Plant Height
QTL	Quantitative Trait Loci
QQ	Quantile-quantile

RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
REC	Recovery
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SEEDL	Seed Length
SEEDT	Seed Thickness
SEEDW	Seed Width
SG	Stem Greenness
SNP	Small Nucleotide Polymorphism
SSR	Simple Sequence Repeats
SVD	Single Value Decomposition
TSWT	Total Seed Weight
WTN	Wilting

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GENERAL ABSTRACT

A considerable set of underutilized crops perform better than their more accepted counterparts when cultivated in less nutritive soils and grossly unfavorable environmental conditions. This advantage necessitates the development of these special crops for the good of sustainable agriculture. Based on the effect of a changing climate and the expected population increase, it is necessary to find a means of improving crop diversities and yield. Less valued crops need to be accepted and incorporated into the food system. Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is one such crop with so much promise of help in enhancing food security. There is a need to find approaches to improve crop yield and stop this decline in food availability, especially in the less-developed world. Bambara groundnut (BGN) and other legumes with the basic compositions of required nutrients such as proteins, oils, and carbohydrates have been major sources of food for humans and animals alike. One of the ways used in selecting crops and improving yields is the application of multi-environment trials (MET) which have been employed in various crops to select the best cultivars that adapt well to various environments. This has aided in the development of many adaptive and stable cultivars across environments. More importantly, advances in next-generation sequencing (NGS) technologies coupled with improvements in bioinformatics tools have strengthened research in plant breeding to tackle food security. The use and accuracy of molecular breeding has been improved through marker-assisted selection (MAS), genotyping, and gene editing, among others. Improvements in genotyping by sequencing (GBS) have been widely successful in crops with reference genomes. Most less-studied crops do not have a reference genome yet, but other approaches can be employed, such as genome-wide association studies (GWAS), quantitative trait loci (QTL) analysis, and comparative genomics, among others.

In this study, a set of 95 accessions of Bambara groundnut that have not been DArT-characterized were selected from the germplasm collection in the IITA Gene bank. These accessions were

evaluated for morphological traits in a MET in 2018 and 2019 in Ibadan and Ikenne, South-West Nigeria. Ibadan is in the derived savanna and Ikenne is in the tropical rain forest. To validate their ability to enhance food and nutrition security, their nutrient, antinutrient, mineral components, and stress responses were accessed. The objectives of the field trials were to evaluate the diversities in the phenotypic and agro-morphological traits in the selected accessions, to examine the effect of the environment on the individual traits and accessions, to discover the most stable and adaptable accession in terms of yield among the selected accessions, and to select the best environment for the crop. Experiments were laid out in a randomized complete block design (RCBD), replicated three times. The plot area was 3m2 with 10 plants per plot. Spacing between each plant was 0.3m and inter-plot spacing was 1m. An alley of 1m separates each replicate. The plants were rainfed and irrigated as appropriate and all standard agronomic practices were observed. After planting, young leaves from 2-week-old plants were collected and DNA was extracted for DArT sequencing. Data were collected from the fields at the appropriate time using the field book. For the nutrient and antinutrient components, good-looking seeds were selected after harvest and analyzed in the Food and Nutrition laboratory. Drought assessment was carried out in the screen house in IITA, Ibadan. Wooden boxes were used and arranged using RCBD in three replicates. Five accessions were planted in each box with 6 plants per accession which were later thinned to 3 after 2 weeks. The boxes were irrigated to field capacity for 24hrs before planting and the moisture content at field capacity was recorded. After planting, watering was done regularly for 4 weeks when plants were fully established and the watering was stopped. Individual plants were scored for wilting, stem greenness, chlorophyll content, and leaf senescence. Scoring was done on days 7, 10, and 13 before watering was resumed. Boxes were watered to field capacity on the day of resumption of irrigation, thereafter once every 2 days for 2 weeks until the experiment was concluded. The collected data were subjected to ANOVA, and the means were separated using the Fischer LSD test. Principal component analysis (PCA), correlation, and cluster analysis were also

evaluated for the different traits. Furthermore, a genome-wide analysis study was conducted on the stress-treated plants to identify genomic regions and candidate genes regulating the stress-response traits studied. The Eberhart and Russell method and GGE biplot were used to analyze the stability analysis and predict the best genotypes and best environment.

Results showed that location was highly significant for all the traits ($p < 0.0001$) except for plant height and leaf length. The accessions varied significantly in plant height, leaf length and width, chlorophyll content, number of petioles, germination count, petiole length, number of pods per plant, number of seeds, hundred seed weight, seed length, seed width, seed thickness, and yield ($p < 0.0001$) while days to flowering ($p<0.001$) and days to 50% germination and total seed weight ($p < 0.01$) were also significant but their responses to the trait days to emergence was not significant. The interaction effect of location and accession was highly significant ($p < 0.0001$) on leaf width, chlorophyll content, number of petioles, germination count, number of pods, number of seeds, and yield, while plant height was also significant at $p < 0.001$, leaf length and seed length were significant at $p < 0.01$, and seed width was significant at $p < 0.05$. However, the interaction between accession and year was highly significant for plant height, leaf width, number of pods, and number of seeds ($p < 0.0001$) and leaf length ($p < 0.001$). There was a highly significant effect of location, accession, and year interaction on leaf length, leaf width, petiole length, number of pods, and number of seeds ($p < 0.0001$), plant height and days to flowering ($p < 0.01$), and hundred seed weight ($p < 0.05$). This implies that high levels of variability and heterogeneity exist among accessions, locations, and years in response to the traits scored. Principal components 1 (24.67%) and 2 (17.63%) account for 42.3% of the total variance observed. Among the variables, seed width (19.53%), seed thickness (19.58%), hundred seed weight (16.98%), seed length (15.93%) and yield (9.76%) were the major contributing traits in PC1, while number of seeds (21.78%), number of pods (18.48%), total seed weight (13.96%), plant height (9.12%), and petiole length (8.93%) were

the major contributing traits in PC2. From the biplot, accessions loading on PC1 are high yielding with thick seeds and long seeds while at the same time having high hundred seed weight. Accessions loaded on PC2 have a high number of seeds, number of pods, and total seed weight. The cluster analysis grouped the accessions into 4 clusters (red, green, blue, and purple) based on the agro-morphological traits with the clusters in red having the highest number of accessions (37 accessions) followed by the ones in green (30), blue having 11, and the purple cluster with 17 accessions. There are lots of significant correlations among the traits scored. In the analysis of the genetic parameters, the phenotypic variance is higher than the genotypic variance in all the traits. Yield (kg ha−1) reported higher phenotypic (19,476.39) and genotypic (5,159.09) variances, while the lower phenotypic (0.68) and genotypic (0.23) variances were observed in leaf width. The traits such as LLE (GCV 7.18, PCV 19.95), GCT (GCV 6.50, PCV 19.61), DTF (GCV 6.31, PCV 10.81), SEEDL (GCV 14.41, PCV 18.19), SEEDW (GCV 11.84, PCV 16.13), and SEEDT (GCV 13.48, PCV 17.66) showed below 20% of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV).

Yield stability analysis showed that seed yield was significantly affected by genotype, environment, and GEI. The mean squares of the accessions were highly significant, also the effect of the environment over the years. Out of the 95 accessions studied, 22 were found to be good-performing and stable, 6 were found to be adaptable, while the remaining accessions were not affected by the environmental factors. The biplot explained 80% of the total variation observed. The first principal component (axis1) explained 48.59% and the second principal component (axis2) explained 31.41%. Accessions TVSu-1866, TVSu-2022, TVSu-2017, TVSu-1943, TVSu-1892, TVSu-2060, and TVSu-1557 were all located at the corners of the polygon in the "which won where" view of the relationship between accessions and environments, indicating that these accessions were outstanding in those environments. TVSu-1706, TVSu-2018, TVSu-1785, TVSu-

1895, and TVSu-1951 accessions performed consistently across all environments. IB2019 was the closest to being an ideal environment, while TVSu-2020 and TVSu-1649 were the most ideal accessions, followed by accessions TVSu-2021, TVSu-1664, TVSu-1866, and TVSu-2025. However, accessions TVSu-1557, TVSu-2060, TVSu-2056, and TVSu-2042 were the worst accessions in terms of yield performance as they are located far from the center of the concentric circle.

The result of the nutrient and antinutrient composition shows a highly significant difference for the traits. The two components account for 41.2% of the total variations observed. The clustering based on the traits depicts four main groups. According to the correlation matrix, protein was significantly correlated with ash, fat, and phytate. Fat correlated with moisture content and tannin; tryptophan correlated slightly with protein content and correlated highly with tannin; moisture content and tannin were also highly correlated. Correlation between drought response traits showed a significant positive correlation between chlorophyll content and recovery. PCA of the traits showed variation in response levels on the three different days that data was taken. Further clustering analysis grouped the accessions based on the response traits.

A total of twenty significant SNPs (considering thresholds of log (p) ≤ 0.001 with $R2 \geq 9\%$) from both the GLM (15) and MLM (5) models were identified by GWAS analysis of the BGN accessions in response to water stress using the Mungbean reference genome. In the study, twelve SNPs associated with drought stress response were identified. These SNPs are co-localized with the *Vradi07g31020*, *Vradi05g01630*, *Vradi06g04840*, *Vradi06g03310*, and *Vradi04g08510* genes which encode for a transaldolase, pectin esterase, proline transporter 1, GDSL esterase, and outer plastidial membrane protein porin respectively. As well as the *Vradi03g05310*, *Vradi10g10930*, *Vradi03g08520*, and *Vradi02g06260* genes encoding for cell division cycle 20.2- cofactor of APC complex, putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase, UDP-

glycosyltransferase 76F1, and SHUGOSHIN 2 respectively. Finally, the genes *Vradi04g01720*, *Vradi02g02440*, and *Vradi07g10090* were discovered, which encode the origin of replication complex subunit 1A, alanine--tRNA ligase, and salicylic acid-binding protein 2.

This study showed that BGN can help improve food and nutritional security, and the accessions used can serve as a source of parent lines for improved varieties.

Keywords: agromorphological traits, Bambara groundnut, candidate genes, food security, Genetic diversity, genotype-by-environment interaction, GWAS, multi-locational trial, marker-trait association, nutritional security, quantitative trait loci, SNPs, sustainability, water-stress response, yield stability

PREFACE

This thesis is written in line with article format style prescribed by North-West University. Thus, the articles are in the publishable format, while the manuscripts (Chapters 2 and 3, which have both been published in **Frontiers in Plant Science** and Chapter 4 which has already been published in **Agronomy** journal) and other chapters (Chapters 5 and 6, which have been submitted for publication to **Journal of Food Composition and Analysis**, and **Scientific Reports** respectively) are written according to the authors instructions of the respective journals. As required by North-West University, contributions of authors for each article/ chapter as well as their accent for use as part of the thesis are provided in Table A.

This thesis contains the following chapters:

Chapter 1 – General introduction: **Harvard (only for referencing style)**

Chapter 2 – Article 1 (Published): **Frontiers in Plant Science (Frontiers)**

Chapter 3 – Article 2 (Published): **Frontiers in Plant Science (Frontiers)**

Chapter 4 – Article 3 (Published): **Agronomy (MDPI)**

Chapter 5 – Article 4 (Submitted): **Journal of Food Composition and Analysis (Elsevier)**

Chapter 6 – Article 5 (Submitted): **Scientific Reports (Springer)**

Chapter 7 – Conclusions and Recommendation: **Harvard (only for referencing style)**

Chapters 1 and 7 were prepared accordingly following the Harvard reference format. The link to instruction for authors for each selected journal is available in Appendix B. Finally, the proofs of submission of articles 1, acceptance of article 2, submission of article 4, and article 5 are provided in Appendices C, D, E, and F respectively.

CHAPTER 1

General Introduction

1.1 Background information

Food demand exceeds available food because of the increasing population. This will lead to an outbreak of famine, especially in the developing world, if the trend continues. Yield-improvement programs set up to solve this problem have not been as effective as expected because the rate of population increase is very high compared with the rate of food production. Therefore, the availability of food having high nutritive and caloric values is essential for human existence.

Today, there is a definite reduction or loss in crop diversity, partly due to the inability to domesticate wild species and the effect of climate change. According to Ulian et al. (2020), there are at least 7,039 edible plant species out of which rice, wheat, and maize are the leading food crops in the world out of the thousands of cultivable crop species available (Kumar et al., 2018). Plant breeding has resulted in the domestication of a few limited wild varieties, development of only a few adapted populations and the selection of a limited number of genotypes (so-called "best genotypes"). Despite these plausible effects, problems of limited diversity in cultivated crop species have risen since most breeding technologies have focused more on the major crops. Therefore, breeding programs should be extended to non-major and underutilized crops to supplement the already major crops.

The objective of plant breeding in an underutilized crop is to increase both intra- and interspecific diversity by exploring these crops for development (Bavec et al., 2017). Most underutilized crops are adapted to specific agro-ecological areas where they have been adapted to withstand biotic and abiotic stresses, thereby contributing in a way to their sustainable production (Cullis and Kunert, 2017). These attributes make them valuable in crop genetic resources as they portray and convey diverse genetic traits. The genetic resource evaluation of a crop for effective breeding should have

the following parametric bases: agronomic value, outcrossing, and yield potential. However, specific trait improvement and successful cultivation of the improved crops are a valuable way of conserving genetic resources for future use. Globally, food security has largely depended on genetically improved crops even though there was an estimated loss of 75% between 1900 and 2000 in crop production (FAO, 2011).

Given the increase in the world population, the production of sustainable food supplies will be a critical challenge in the twenty-first century. The world population is projected to reach 9 billion by 2050 (Gerten et al., 2020, McKenzie and Williams, 2015), indicating that food supplies must be doubled to meet the needs of the expanding population (Laplaze et al., 2018, Kummu et al., 2017). Apart from increasing the quantity of food, improving quality is also critical to maintaining nutritive values with increased potential for yield. With the issue of underdevelopment and climate changes, there is no better way to combat the looming food scarcity than to look within. Therefore, the improvement of indigenous crops will offer a lasting solution in a continent such as Africa.

Legumes, which are the most important food crops behind cereals, belong to the family *Leguminosae*. They are important sources of proteins and minerals, which makes them essential for poor people in underdeveloped communities, especially in Africa and Asia, where the majority cannot afford meat and fish. Utilization of legumes in combatting malnutrition and food insecurity (Ojiewo et al., 2015, Mubaiwa et al., 2018) has been a focus of research in most developing countries. This outlines the visible potential (drought tolerance, thriving in marginal soils, nitrogen fixation, and acceptable nutritional value) that needs to be exploited in these crops. Areas where they are mainly grown include Nigeria, Senegal, Togo, Indonesia, Cameroun, India, and Côte d'Ivoire (Borget, 1992). They are classified as pulses (Bambara groundnut), oilseeds (Soybean and Groundnut), forage legumes (Winged bean), tuberous roots (Yam bean), and food crops (Cowpea) (Foyer et al., 2016, Mayes et al., 2019). Some are well known and extensively incorporated into

the global food system, while some are still relatively underutilized or unknown. Underutilization can be due to a lack of knowledge or information on their uses, and some are hard to cook. Among the underutilized legumes are African yam bean, Pigeon pea, and Bambara groundnut (BGN).

Apart from the widely known legumes, a subset of the underutilized legumes performs comparatively better in marginal soils and under less favorable environmental conditions than other major crops. Hence, developing these subsets further for future agriculture makes a suitable and complementary approach to the continued use of major crops. This is particularly important given the expected negative impact of climate change on current major crop production systems and the gap between the current rate of genetic improvement of most major crops and the higher rates required to be able to feed the predicted nine billion people in 2050 (Laplaze et al., 2018, Lewandowski et al., 2018).

One such underutilized crop, Bambara groundnut (*Vigna subterranea* (L.) Verdc.), is an indigenous African legume that thrives in hot climates and is well suited to poor, infertile soils where other crops do not produce reasonable yields (Atoyebi et al., 2013). It is classified under the family *Fabaceae*, sub-family *Faboidea,* and the genus *Vigna* (Bamshaiye et al., 2011). There are two botanical varieties known; the wild botanical variety (*Vigna subterranea* var. *spontanea*) and the cultivated botanical variety (*Vigna subterranea* var. *subterranea*) (Yao et al., 2015). These varieties are diverse in their morphology, nutritional components, responses to biotic and abiotic factors, and so on. This legume seed crop was reported to originate from West Africa, from the Bambara district near Timbuktu (Jideani and Diedericks, 2014). It is now widely grown in Africa, Malaysia, South and Central America, some parts of Northern Australia, Sri Lanka, and Indonesia (Azam-Ali et al., 2001). Different places have their indigenous names for BGN. For example, it can be called Madagascar groundnut, Baffin pea, Voandzou, Indhlubu, underground bean, and Nzama in Malawi, In Nigeria it is Epa-Roro, in South Africa Jugo beans, and in Zimbabwe Nyimo beans

(Bationo et al., 2011, Vijaya, 2011, Oso et al., 2013, Nandini, 2016, Emelike and Barber, 2018, Jideani and Diedericks, 2014). One of its most important traits is its ability to thrive in adverse environmental conditions and nutrient-poor soil where other crops might not thrive. Another characteristic is its ability to be intercropped with other crops such as Maize, which helps in providing nitrogen for the Maize through nitrogen fixation (Alhassan and Egbe, 2014, Abate and Alemayehu, 2018).

Its ability to fix nitrogen improves soil fertility and makes it useful in crop rotation. This provides the possibility of a crop being grown without the use of expensive chemicals and fertilizers. Bearing in mind that chemicals and fertilizers are usually difficult to obtain in isolated areas, therefore, nitrogen fixation by BGN adds to its advantages for farmers (Cleasby et al., 2016, Musa et al., 2016). Like Peanuts, the BGN plant grows close to the ground with seeds forming underground. Its maturity rate is between 3-6 months. This crop has also shown large diversity in its genetic resources for improvement (Aliyu et al., 2016, Mayes et al., 2019).

Linnaeus in 1763 classified the crop in the *Plantarum* specie naming it *Glycine subterranea*. In 1806, Du Petit–Thouars proposed the name *Voandzeia subterranea (L). Thouars* after discovering it in Madagascar (Young, 1978). This name was widely used for over a century. *Vigna subterranea* belongs to the genus *Vigna*, subclass *Phaseolinae*, class *Phaseoleae* and family *Papilionaceae* (Bamshaiye et al., 2011, Azam-Ali et al., 2001). As stated earlier, it was known as *Voandzeia subterranea* for more than a century before it was changed to *Vigna subterranea* in 1980 (Borget, 1992). A comprehensive botanical study by Maréchal et al. (1978), found striking similarities between BGN and plant species of the genus *Vigna* leading to the studies by Verdcourt who later proposed the name *Vigna subterranea* (L) Verdc (Goli, 1995).

BGN is a small herb having both prostrate and erect forms and grows to a height of 0.30m to 0.35m. Like the Groundnut, it has compound leaves of three leaflets. The plant is characterized by

either bunched leaves which are self-pollinating, forming a crown on the soil surface, or spreading leaves which are cross-pollinating. The branched stems of the plant root at the nodes to form a bunched herbaceous annual with a thick taproot which forms a profusion of lateral roots towards its tip (Al Shareef et al., 2014). The stem branches about one week after planting, and a considerable number of branches are produced, giving the plant a bushy appearance. It is made up of about ten running stems with very short internodes, with roots growing from nodes at each stem. The leaf arrangements and conformation are alternate and trifoliate. It possesses auxiliary peduncles which elongate from the stem nodes, each of which has one to three flowers. The plant is literally considered to be autogamous (Molosiwa et al., 2015, Aliyu et al., 2016) with pale-yellow flowers which are borne on the branching stems. After the occurrence of fertilization, the stem grows into the soil with the already developed seed. The pod, which is about 1.25–2.5cm in diameter, ends up about 1cm beneath the soil surface, containing one or two seeds formed after 40 days of fertilization (Gibbon and Pain, 1985). The pods, which are either green or purple at maturity, cluster around the center and the secondary roots.

On the field, plant development and productivity are controlled by various extreme environmental factors such as drought, heat, salinity, cold, or pathogen infection, which may delay or reduce seed germination, reduce seedling growth, and decrease crop yields. Finding a lasting solution to these problems has been a major current research interest. In this quest, the crop genome sequence is a key factor for understanding the processes, both physiological and biochemical, controlling plant traits and their mode of responses to biotic and abiotic environmental stresses. The rapid evolution of genome sequencing technologies has resulted in the generation of large genomic data sequences of plant genomes, creating an opportunity for the application of this technology to crop improvement (Yuan et al., 2017, Zargar and Rai, 2017). In the vastly improving area of life science technologies, new areas have merged for elucidating gene functions and metabolic pathways; and

'omics' technologies coupled with improved bioinformatics tools and databases. As our understanding of the key processes increases, it must be translated into research in plant development and improved crop yield.

Various environments affect different genotypes diversely. A genotype behaves differently when subjected to different environments. Thus, coupled with individual crop genetic diversity, each environmental condition such as water availability, soil composition, soil type, temperature, rainfall pattern, humidity, photoperiod, etc., affects the production outcome of a crop. Hence the importance of studying genotype-environment interactions on crop production in a multi environment trial (MET). As a result, GGE biplot analysis (Yan et al., 2007, Yan and Tinker, 2006) is one of the two most common methods used for this purpose. Analysis of MET output using GGE biplot helps to determine the best environment for a crop, the best accessions of a crop in each environment and in all environments under consideration, and the most stable accession across all environments studied. With this, the best selections can be made for improved varieties. GGE biplot analysis of yield stability for Andean dry bean accessions grown under different abiotic stress regimes in Tanzania was reported by Mndolwa et al. (2019). Soybean performance and stability in MET using GGE biplot analysis was also reported by Dalló et al. (2019). Other applications of GGE biplot have been reported on various crops such as Maize (Badu-Apraku and Akinwale, 2019), Sugarcane (Tena et al., 2019), Sunflower (Ahmed et al., 2019), Rice (Oladosu et al., 2017), and Wheat (Buenrostro-Rodríguez et al., 2019).

Furthermore, various next-generation based technology protocols have been developed for large data sets which have been applied in genome-wide association studies (GWAS), quantitative linkage locus (QTL) analysis, linkage mapping, genome selection, population genetics, and SNP detections (Singh et al., 2017, Singh et al., 2011). All these protocols have been rightly optimized and modified for several model crops for various traits such as water stress (Ayalew et al., 2018),

growth and yield traits , salinity tolerance, nutrient, etc. There are no improved varieties of BGN yet, which means that the crop is cultivated from local varieties. The genetic diversity observed in the wild type is not comparable to that observed in domesticated types (Zhang et al., 2019). The ability of breeding programs to harness the advantages of the new life science technologies which apply various molecular markers to gain a better understanding of BGN genetics provides a great step in the right direction. Due to the importance of this crop, these technologies can improve farmers' income through improved crop yield as well as improving both global and local food security.

In addition, the use of molecular markers has immensely improved plant breeding. Various markers have been utilized on various crops for selections and improvements. One such molecular marker is the diversity array technology (DArT) markers, which have been used in various crops of importance (Fayaz et al., 2019, Mogga et al., 2018, Dracatos et al., 2019). SNPs for important crop traits are identified through molecular markers. In the absence of reference genomes, DArT markers are reportedly of great importance, hence their increased interest in the genetic studies of underutilized crops. Other markers such as RFLP, AFLP, ISSR etc. are also used in various studies involving major and underutilized crops. Further explanation of these markers is in chapter two of this thesis.

Finally, the ongoing efforts to improve crop species' tolerance to biotic and abiotic stresses, as well as maintaining high levels of productivity, profitability, and quality, involve ongoing activities focused on genetic resource conservation, as well as crop species improvement. Effective breeding trait selection is heavily reliant on accurate estimates of the heritability of morphological and agronomical traits. The use of molecular markers for these traits' aids in the introduction of new cultivars and accelerates agricultural progress. The fact that heritable variation is found in populations means that this heritability can be used to estimate how much a crop can be improved

by selecting for these traits. Heritability is the genetic link between breeding and phenotypic values (Falconer et al., 1996). That is, it is a reliable breeding predictor of phenotype value. In other words, it measures how much of the phenotype will be passed down to the next generation. A direct link exists between heritability and selection response. Hence, the magnitude of the selection procedure influences heritability, as well as genetic gain and the degree of impact of genes. High genetic advance and high heritability show that the traits can be selected for improvement and they are not affected by the environment (Padmaja et al., 2008, Dhivya et al., 2014).

1.2 Problem Identification

There is a limited number of plant species that are used as food source. This has limited the availability of food to feed the ever-increasing population. Furthermore, climate change, increasing population and urbanization has further hindered crop production and availability. The rate at which population is increasing is exceeding the rate of food production for feeding the world. Urbanization is also reducing the availability of land for growing food, as productive land is taken up by buildings and industries. In addition, limited diversity in available food crop has called for urgent alternatives to these major crops to ensure food security. Hence the advocacy for marginalized/underutilized/orphan crops to support the major food crops. Underutilized crops reportedly thrive in marginalized soils where these major crops cannot thrive. Their ability to survive climate change impacts such as drought, heat, salinity stress more than the major crops is an advantage. If these crops can produce favorably without in-depth research, then we can imagine what and in-depth research input and focus on these crops will achieve in food security and human sustainability.

1.3 Justification of the study

Improved BGN productivity is required to validate its use in varied agricultural systems. Understanding BGN's physical and physiological traits is the first step to efficiently using its genetic diversity. This method will also benefit breeders and farmers by providing critical baseline

data on known landraces. Evaluating landraces' features can assist breeders characterize valuable germplasm and improve selection efficiency for specific environmental situations. Thus, morphological, physiological, and genetic variations influencing growth, development, yield, and nutritional content of BGN landraces must be characterized. Hence, the need to improve and promote the crop for the enhancement of food security, especially in sub-Saharan Africa. The work in this study is further justified by the following research questions:

- Do variations exist between BGN accessions in response to morphological traits?
- Is BGN yield affected by the environment?
- What are the genetic bases of the observed agro-morphological variations in BGN?
- What is the response of BGN to water stress using molecular and phenotypic data?

1.4 Objectives of the study

1.4.1 General objective

This research aims to identify and harness the treasures locked in the BGN and to identify genetic diversity in some selected accessions to unearth the potential of this underutilized crop in combatting food security through the application of NGS technologies. Furthermore, parent lines for BGN breeding programs can be identified from the outcome of this study.

1.4.2 Specific objectives

Simply, the specific objectives of this study were:

i. To characterize 95 different BGN accessions using morphological and physiological differences influencing their growth, development, and yield under field conditions to select superior landraces for production.

ii. To assess nutritional and anti-nutritional contents among the 95 BGN accessions to identify accessions with high content of multiple nutrients.

iii. To assess genetic variation and relatedness for drought stress response among BGN accessions using DArT markers as a preliminary step towards BGN improvement.

iv. To identify putative candidate genes for drought tolerance as a preliminary study for improved BGN breeding program.

1.5 Hypothesis

i. The BGN accessions were diverse in agronomic performance and physiological traits.

ii. The BGN accessions consisted of genetically divergent individuals both in agronomic performance, physiological traits, and drought stress response.

iii. The BGN accessions are diverse in their nutrient and antinutrient components.

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CHAPTER 2: ARTICLE 1

Breeding potentials of Bambara groundnut for food and nutrition security in the face of climate change

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Breeding Potentials of Bambara Groundnut for Food and Nutrition Security in the Face of Climate Change

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Constant production of quality food should be a norm in any community, but climate change, increasing population, and unavailability of land for farming affect food production. As a result, food scarcity is affecting some communities, especially in the developing world. Finding a stable solution to this problem is a major cause of concern for researchers. Synergistic application of molecular marker techniques with next generation sequencing (NGS) technologies can unlock the potentials hidden in most crop genomes for improving yield and food availability. Most crops such as Bambara groundnut (BGN), Winged bean, and African yam bean are underutilized. These underutilized crops can compete with the major crops such as cowpea, soybean, maize, and rice, in areas of nutrition, ability to withstand drought stress, economic importance, and food production. One of these underutilized crops, BGN [*Vigna subterranea* (L.), Verdc.], is an indigenous African legume and can survive in tropical climates and marginal soils. In this review, we focus on the roles of BGN and the opportunities it possesses in tackling food insecurity and its benefits to local farmers. We will discuss BGN's potential impact on global food production and how the advances in NGS technologies can enhance its production.

Keywords: climate change research, food security, next generation sequencing, Bambara groundnut, underutilized legume, water deficit stress

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INTRODUCTION

The evidence of climate change is now overwhelming, with a rise in global temperature predicted by up to 4°C by 2100, with alterations in wind patterns, and precipitation (Thuiller, 2007). According to the United Nations, it involves shifts in weather patterns which affect food production, and rises in sea levels which result in flooding.[1] Climate change is a danger to the sustainability of food and nutrition. It is one of the most pressing challenges facing food security and nutrition (Abberton et al., 2016). Improving crops for adaptation to climate change effects is essential to avert a looming decline in food production. Hence, the need to develop crops that are resistant to drought stress, salinity stress, higher or colder temperatures, and flooding, which are made more pronounced by climate change.

[1] https://www.un.org/en/climatechange/what-is-climate-change

The importance of food security in society cannot be overemphasized but, achieving food security is hampered by climate change impact. According to the World Food Summit (1996), food security exists when sufficient safe and nutritious food is available for people. These foods must also meet the relevant dietary needs to maintain a healthy life. Household food security is applying this concept to the family level, with individuals within households as the focus of concern. However, most households in the developing world do not have daily access to quality food. The FAO, therefore, deemed food security to be achieved based on these four pillars; availability, accessibility, sustainability/stability, and utilization (**Figure 1**).

One of the highest occurrences of food insecurity and malnutrition is recorded in Africa (Khan et al., 2016). Africa and Asia have been postulated to have the highest population increase by 2050 because most urban growth will be concentrated on these two continents (Huang et al., 2019). This will place severe strain on the available food system; therefore, there is the need for an extensive revolution in agricultural research to mitigate the effect of climate change on food availability in these regions. In a bid to also achieve food availability, some crops which are domesticated in their locality are grown, especially in Africa and some parts of Asia. However, these crops are not widely accepted when compared with their major counterparts. Therefore, they are consumed primarily within their locality. These crops are termed orphan/underutilized/neglected crops grown by subsistence farmers in their local adaptations. They have proven to be vital sources of nutrients and income (Ratnayake et al., 2020), even when faced with the impacts of climate change.

The development of a high-yielding and climate-resilient crop is essential for food security. Although there have been positive improvements from conventional plant breeding, the rate of genetic gain has not been entirely up to expectations (Chen L.Y. et al., 2019; Piunno et al., 2019). Both conventional breeding (such as grafting and crossing techniques) and new technologies [such as genome editing, epigenetics, phenomics, transgenesis, protoplast fusion, and marker-assisted selection (MAS) breeding] can be harnessed to mitigate the impact of climate change on food availability. Furthermore, increased diversity in cultivated plant species will improve the plant germplasm and increase genetic coverage for improvement. Despite the availability of over 31,000 useful plant species, it is surprising that just three crops (rice, wheat, and maize) provide over 50% of plant-derived calories for use (Borrell et al., 2020). However, the custom of planting limited species of a crop is not favorable because these crops can become vulnerable to climate instability. Besides, more crop varieties will allow variability and diversity in improving important adaptive mechanisms and nutritive values, while keeping the present varieties to prevent loss of diversity. Agricultural biotechnological research for the development of more crop varieties has been an important focus area of the Consultative Group on International Agricultural Research (CGIAR) in collaboration with other partners (Hickey et al., 2017). Researchers carried out several collaborative types of research on different staple food crops in various developing countries. These researches, especially those involving the locally adapted underutilized crops, improve the lives of the farmers in these countries.

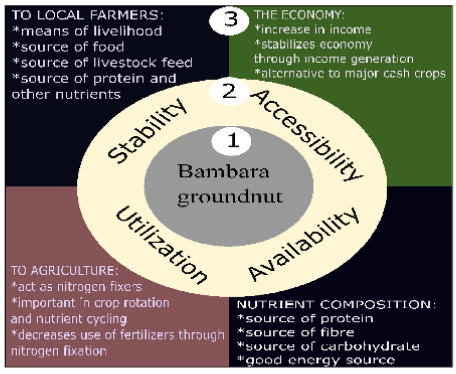

FIGURE 1 | Aligning BGN with the SDG goals. The relationship of Bambara groundnut (1) to the four pillars of food security (2) and the importance of this crop to farmers, agriculture, the economy, and its nutrient composition (3).

Due to the predicted increase in the world population, there is the need for an immediate revolution in the agricultural system to satisfy food demand. With underdevelopment and climate change, there is no better solution to combat the looming food scarcity than to look within. Therefore, the improvement of underutilized indigenous crops could proffer a lasting solution to food insecurity in Africa. The best potential underutilized crop must be able to adapt to its environment, have market value, acceptable taste and texture, and must be able to thrive well with less agricultural inputs (Mayes et al., 2011).

Bambara groundnut (BGN) is an underutilized crop with a lot of potentials for achieving food security in sub-Saharan Africa. Africa produces ∼0.3 million tons of BGN annually with an average of 0.85 t/ha. Nigeria, Burkina Faso, and Niger used to be the largest producers in Africa producing 0.1 million, 44,712, and 30,000 tons, respectively (Hillocks et al., 2012), but recently Burkina Faso, Niger, and Cameroon are the largest producers with 74% of the world production (Majola et al., 2021). The lipid content in BGN compares favorably well with that observed in cowpea (1–1.6%), pigeon pea (1.2–1.5%) but lower than groundnut (45.3–47.7%) (Azam-Ali et al., 2001). The predicted yield in Africa is between 300 and 3,000 kg/ha with majority of west African countries predicted to produce in the range of 300–1,000 kg/ha while southern African countries were predicted to produce between 1,000 and 3,000 kg/ha (Azam-Ali et al., 2001). Hence, the highest production of the crop should be from the southern part of the continent. This view is confirmed by Majola et al. (2021), who reported the southern African region as the suitable region in the continent for the production of BGN. Despite its reported qualities, one of the critical challenges facing BGN production and other underutilized legumes is their low yield when compared to the major crops (Sidibé et al., 2020; Temegne et al., 2020). This can result from loss in genetic diversity during domestication, as most of their wild relatives

perform better and adapt well over the years (Zhang et al., 2019). But strategic technological advancement in the production of BGN involving genetic analysis can provide essential data for breeding programs that will enhance its potential in improving food and nutritional availability in the presence of low water availability. Furthermore, when other legumes, such as soybean, chickpea, and groundnut, are shifting into the post-genomic era, BGN is just drifting into its genomic era. As a result, available genetic resources for efficient breeding programs are limited.

Hence, this review will be looking at the current knowledge in molecular breeding through next generation sequencing (NGS) technologies, and the progress expected from these technologies on BGN production. We will take a brief look into some of the different NGS technologies that apply to improving crop yield. Each has its specific attributes depending on the research questions to be answered, such as trait mapping and association analysis (Liu and Cheng, 2020), breeding for drought tolerance (Nithya et al., 2020), breeding for salt tolerance (Yu et al., 2020), and genome diversity (Hassani et al., 2020). Finally, we will outline some key points that need to be addressed for BGN to become fully utilized in improving food and nutrition security.

BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.) PROVIDES A SUSTAINABLE ALTERNATIVE TO MAJOR CROPS

Bambara groundnut is gradually receiving more international research attention. It is considered a complete food containing a high amount of protein and other nutrients (Halimi et al., 2019; **Table 1**). A pulse with a subterranean fruit set, cultivated by subsistence farmers mostly in the semiarid part of Africa, and an African legume (Mayes et al., 2019) with variations in seed color and morphology: such are the attributes of BGN (**Figure 2**). The botanical name of the crop is *Vigna subterranea* (L) Verdc, which consists of the wild species type (*V. subterranea* var. *spontanea*) and the cultivated variety (*V. subterranea* var. *subterranea*). Its high carbohydrate amount and considerable protein contents make it regarded as a complete food (Bamshaiye et al., 2011).

Bambara groundnut is believed to be the most resilient to drought among grain legumes (Ntundu, 1997; Jørgensen et al., 2010). Wild varieties predominate due to limited research into domesticating new varieties. The major producing/exporting countries are Niger, Ghana, Chad, Nigeria, Mali, Senegal, Côte d'Ivoire, Burkina Faso, and Togo. The International Institute of Tropical Agriculture (IITA), also in Nigeria, holds over 1900 accessions obtained from various countries in their Genetic Resources Center (Paliwal et al., 2020). Knowledge of the genetic variation of BGN accessions will be important for their efficient use in breeding program, studies on the crop's evolution, and conservation purposes. BGN is characterized by a high degree of variability for various morphological, physiological, and agronomic traits. To analyze the genetic structure of crop germplasm, the estimation of variation within and between populations of its species is important. Genetic diversity on the crops has been studied using various molecular markers (Olukolu et al., 2012; Fatimah and Ardiarini, 2018; Konate et al., 2019), and more researches are still ongoing in this regard. As landraces are not available, BGN varieties must still be developed; thus, their continued improvement and development contributes to the development of diverse, superior varieties. Mutant collections made using the tools of genetic engineering can supply new genetic diversity as well.

Bambara groundnut belong to the genus *Vigna*, subclass *Phaseolinae*, class *Phaseoleae* and family *Leguminosae* (Azam-Ali et al., 2001; Bamshaiye et al., 2011). It was known as *Voandzeia subterranea* for more than a century before it was changed to *V. subterranea* in 1980 (Borget, 1992). A comprehensive botanical study by Maréchal et al. (1978), found striking similarities between BGN and plant species of the genus *Vigna* leading to the studies by Verdcourt who later proposed the name *V. subterranea* (L) Verdc (Goli, 1995).

TABLE 1 | Nutritional components of BGN and some underutilized legumes.

Nutrient components	BGN	Cowpea	African yam bean	Winged bean	Mung bean
Moisture (%)	4.30	10.79	8.84	5.55	8.08
Protein (%)	23.59	26.76	21.26	28.52	26.50
Carbohydrate (%)	64.4	50.53	61.92	34.11	56.52
Fat (%)	6.50	0.92	1.76	16.72	1.33
Fiber (%)	5.49	11.03	5.19	5.51	3.67
Ash (%)	4.30	3.12	3.40	4.56	3.91

Source: BGN (Yusuf et al., 2008; Halimi et al., 2019); Cowpea (Gondwe et al., 2019); African yam bean (Adegboyega et al., 2020); Winged bean (Adegboyega et al., 2019); and Mung bean (Li et al., 2010).

MAINSTREAMING BAMBARA GROUNDNUT FOR ENSURING FOOD AND NUTRITIONAL SECURITY

Bambara groundnut plays an important role not only in addressing food and nutritional well-being, but also in boosting immunity (Adebowale et al., 2011; Adedayo et al., 2021), livestock feed (Feldman et al., 2019), improving biodiversity, and protecting farmers' livelihoods. Resistant starch is concentrated in this crop, which encourages the slow and sustained release of glucose into the bloodstream, making a healthy diet (Cummings and Englyst, 1995). These characteristics highlight the significance of BGN being introduced into the mainstream. Prioritizing the development of the best agronomic practices, improving storage and supply chains, and using better methods of delivery for BGN is possible, but it will necessitate developing a roadmap that includes developing beneficial traits, utilizing better agronomic practices, and optimizing supply chains. Identifying suitable BGN species for cultivation and providing training to farmers is required at hotspots where other species are being grown.

When searching for an appropriate variety, location, climatic conditions, soil fertility, and availability of human resources

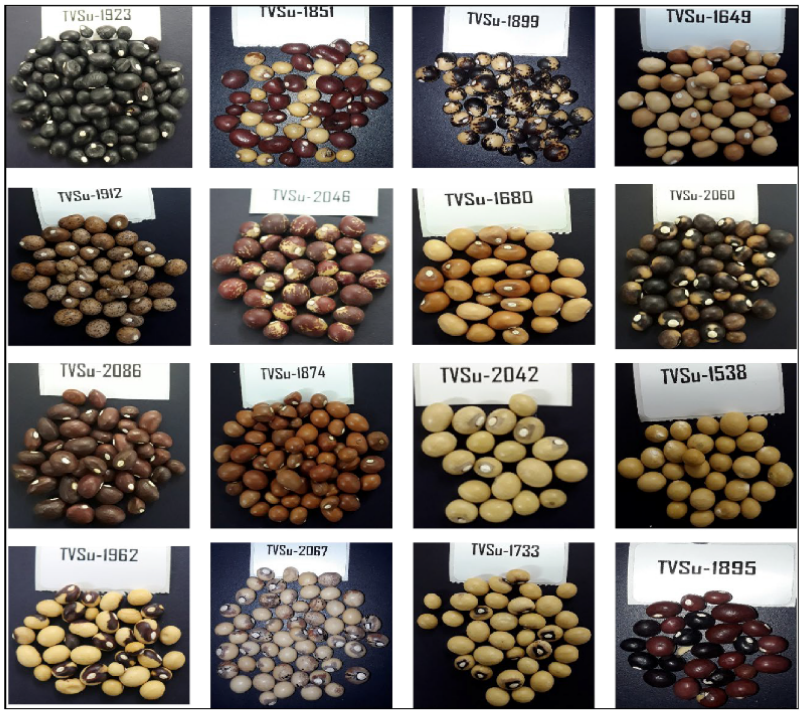

FIGURE 2 | Bambara groundnut seeds showing variability in different accessions through colors, shapes, and eye patterns.

all play a role. If there can be easy access to worldwide germplasm repositories, releasing seed materials for cultivation will be simpler; however, improvements in target location may be required to produce higher agricultural values of the produce. Because of the availability of recent and relevant genetic tools and biotechnological interventions, it would be beneficial to work with modern genetic engineering techniques and biotechnological interventions to aid in the BGN breeding process. Simple crop management techniques, including straightforward irrigation techniques, soil preparation, and crop rotation, help to produce good crops. Using this techniques thus enable higher yields and avoid crop failure (Muthamilarasan et al., 2019). Insect pests that have been observed to be attacking BGN have been reported (Lale and Vidal, 2003; Majola et al., 2021). An understanding of these pests' biology, including their mode of action, and the various host defenses that organisms use to resist insect pests is critical for the development of insect-tolerant varieties. Mitigating the effects of insect pests can also be helped by optimizing integrated pest management strategies.

Large scale production of seed grains can encounter bottlenecks in post-harvest processing and storage, but streamlined processes and equipment are available to minimize processing and storage loss, as is the case with BGN. Crop-specific technologies are required to develop and implement solutions that are specific to each BGN grain type. Long-term storage of BGN grains necessitates the use of protective measures such as storing BGN grain at temperatures far beyond the optimal range to avoid sprouting or rancidity, and as a result, valuable produce gradually turns rancid. This is attributed to the oxidation of the unsaturated fatty acids (Adeleke et al., 2018). To mitigate global hunger and child malnutrition indices, these storage facilities can be assessed by people during natural disasters or future pandemics. Incorporating an established supply chain helps ensure the agricultural outputs, such as BGN, that benefit farmers and stakeholders who are involved in small scale BGN cultivation can be distributed throughout the supply chain.

BIOTIC AND ABIOTIC FACTORS AFFECTING BAMBARA GROUNDNUT PRODUCTION

Diseases and pest infestation on the crop are not very pertinent. Therefore, reports have not been elaborate on it. Ability to

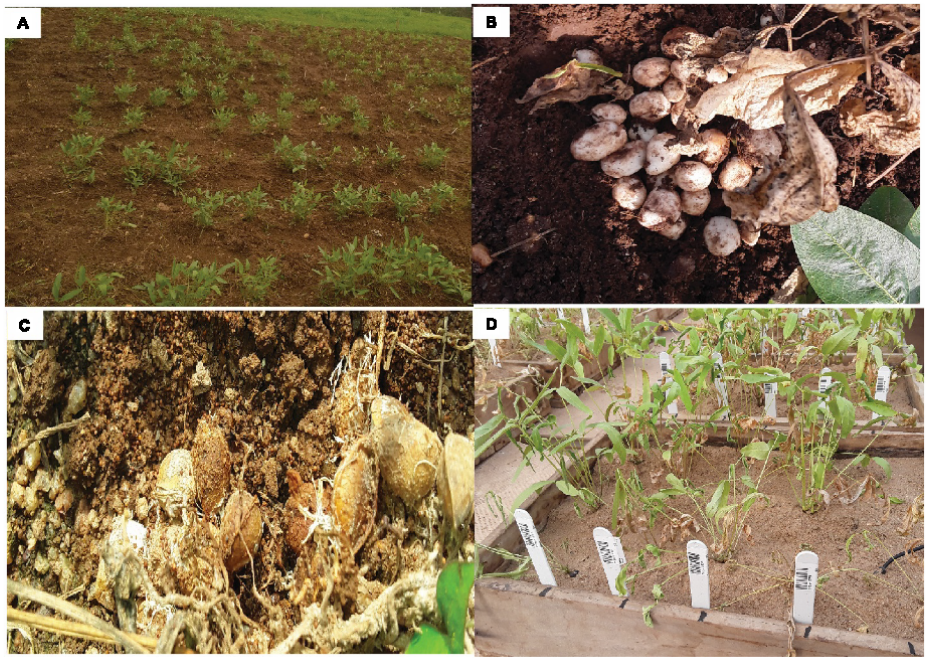

FIGURE 3 | (A) Bambara groundnut (BGN) on the field; **(B)** healthy matured BGN pods during harvest; **(C)** infected BGN pods; **(D)** drought stressed BGN plants.

produce their foods below the soil and the hardness of the seeds aid in their resistance to pests. Notwithstanding, BGN still hosts pathogens and insect pests which cause a significant economic impact through yield loss (**Figure 3**). Major biotic constraints to BGN production are disease, insects, and viruses. There are diverse stands as regards the crop being affected by pests and diseases. Purseglove (1992) stated that the crop is free from pests and diseases meaning that it cannot be affected by any pest or disease. This notion is partially supported by Gibbon and Pain (1985). However, Gibbon and Pain (1985) also reported the possibility of infestation by leafhoppers (*Hilda patruelis* and *Empoasca facialis*). Tanimu and Aliyu (1990) in their own view observed that the crop is free from other insect pests that affect other legumes such as cowpea and peanut. This limits the use of a pesticide when cultivating the crop. Goli (1995) reported the attack of BGN pods by termites. Immature pods can be damaged by moth beetle (*Piezotraachelus ugandum*), while larvae of *Rivellia* causes damage to the root nodules. Reports of insect infestation on the crop mainly during storage has been reported in the works of Kabir et al. (2017); Nyamador et al. (2017), Mahama et al. (2018), and Moussa et al. (2018).

Moving away from insect pests, Brink et al. (2006) reported that BGN can be infested by fungal pathogens resulting in fungal diseases such as cercospora leaf spot (*Cercospora* spp.), powdery mildew (*Erysiphe polygoni*), and Fusaruim wilt (*Fusarium oxysporum*). Root-knot nematode (*Meloidogyne javanica*) attack the crop in sandy soil (Fourie et al., 2017). In addition, viruses such as cowpea mottle virus (Hema et al., 2014), necrotic mosaic virus (Masindeni, 2006), white clover mosaic virus (Adu-Dapaah et al., 2004) and two potyviruses (Mkandawire, 2007) have been reported to affect the crop. When stored while it is damp, molds are able to grow on the seeds thereby permitting weevils to be able to attack the seed. Bruchids (*Callosobruchus maculatus*) are the most important pest attacking the seeds of the crop during storage (Kosini and Nukenine, 2017).

Aside from pests and diseases, various environmental factors also pose some challenges to the yield of the crop. Factors such as temperature, drought, and salt (Suzuki et al., 2014), affects its growth at different developmental stages (**Figure 3**). These factors reduce the yield of crops worldwide by as much as 50%. The stresses resulting from the impact of these factors causes morphological, physiological, biochemical, and molecular alterations in the crop which affects its productivity and yield (Bita and Gerats, 2013). In BGN, biotic stress causes yield reduction, but abiotic stress is the most limiting factor causing unstable yield. Important abiotic stresses are temperature, water, soil conditions, and drought stress.

Soil texture and structure that enhance aeration in the soil determine the suitability of soils for BGN production. The seeds of BGN are borne below the soil surface; therefore, the choice of

soil type is very important. The crop prefers well-drained, sandy loam soil because they can utilize lighter rain showers to greater advantage than clay soil and the soil cannot damage the seeds (Swanevelder, 1998).

RECENT PROGRESS IN BAMBARA GROUNDNUT RESEARCH AND ROOM FOR IMPROVEMENT

Crop improvement depends on access to genetic resources. Genetic resources provide parent lines for characterization in breeding programs. Hence, effective use of these lines is of high importance in improving crops. In BGN, available genetic resources have been utilized in various studies which have resulted in the development of molecular markers for trait mapping and possible improvement. However, the lack of reference genome for BGN has hindered the progress of improvement in this regard. Till date, whole genome sequencing generates molecular data that can be used in comparative genomics analysis between underutilized legumes like BGN and their major crop counterparts to identify genes, alleles, and quantitative trait loci (QTL) for yield-determining, agronomic, and climate-resilience traits [reviewed by Khan et al. (2016)]. This techniques was used by Chai et al. (2017) to identify QTL involved in agronomic and drought related traits using an expression marker-based genetic map based on major crop resources developed in soybean. In another study, Ho et al. (2017) carried out a comparative genomics study based on common bean, adzuki bean, and mung bean genomes to identify conserved genes in BGN. The use of molecular markers has resulted in the development of large scale, genome-wide molecular markers, high-density maps, and genomic regions governing key traits (Ahmad et al., 2016).

The primary goal of BGN or any plant breeding program is to alleviate production constraints. Owing to limited genome data, most genetic traits have received less attention in BGN research. It was notable that only the genetic diversity studies from molecular markers have been studied in BGN. No reported genome wide association study on BGN has been reported while very few have identified genes for some traits using comparative genomics studies (Ho et al., 2017). This research should be expanded to aid in the development of genomics-enabled breeding for the improvement of higher yield and higher nutrient-containing varieties. Another intriguing feature of BGN grain is the presence of resistant starch and its hard-to-cook phenomenon (Oyeyinka et al., 2017; Akintayo et al., 2021), which will require further investigation to uncover its genetics and genomics. In comparison, research on nutrient composition (Hlanga et al., 2021), antinutrient composition (Adegboyega et al., 2021), amino acids composition (Oyeyinka et al., 2019), antioxidant composition, responses to environmental stress (Bonthala et al., 2016; Khan et al., 2017), and photoperiodism (Kendabie et al., 2020), has been conducted in BGN. Achievements that further genomic tools such as molecular markers, gene editing, omics will bring to BGN breeding are discussed elsewhere in this study by comparing the impact of these tools on some major crops.

Key Points to Be Addressed in Bambara Groundnut Improvement Include

Tackling Low Yield Syndrome

Underground pods are a big challenge for the yield output in this crop. The seeds must be covered or else they will not mature. This exposes them to waterlogging during heavy rainfall; it can also expose them to too much heat in hot climates. Because of their high level of oil content, excessive heat is not good for the embryo. This situation can be helped through genotype by environment interaction studies. Accessions should be assessed for their performance in various environments and accessions with the best and stable yield at the various environments could be developed for improved varieties (Olanrewaju et al., 2021). Accurate records of exact maturity dates should also be taken so that the pods will not overstay in the soil before they are harvested. Post-harvest procedures such as threshing and storage pose another great challenge to the output of this crop. There is no mechanized way of threshing; when done manually, most of the seeds are destroyed and a large portion of the harvest is lost. Finding better ways to prevent these significant losses will boost total output.

Research Funding

Until recently, research funding for underutilized crops have been limited when compared with their counterparts among major crops. Most organizations prefer to support widely known crops. The availability of funds for research on these crops will not only reveal their importance, but will also create an opportunity to generate more varieties from their wild counterparts. Domestication will be made easier as the latest advances in technology will open new frontiers. The role of biotechnology in the preservation of germplasms cannot be over-emphasized. Collections of these germplasms allow the selection of traits for improved breeding (Dawson et al., 2009). Therefore, technologies such as gene editing, genomics, proteomics, transcriptomics, and other omics technologies can all help to improve different varieties of BGN for various useful traits such as drought tolerance, salt tolerance, nutrient composition, and physical traits.

Favorable Government Policies

Most countries in the developing world import most of their food. Policies can be set by the government for a gradual reduction in importation. This will lead to the consumption of the local underutilized crops such as BGN until they gradually become fully accepted. An increase in awareness of the benefits of BGN leads to an increase in consumption. Once demand has increased, there will be a definite increase in the cultivation and production of these locally grown crop species. Therefore, there should be a favorable balance between policies governing exports and imports of food crops (Tomlinson, 2013). Supporting exports of the underutilized crops will be a source of revenue for the nation. This will improve the economy and provide another source of income generation.

Crop Popularity Issues

Most people are not aware of these underutilized crops because most of them are grown only in the immediate locality of their use. They are not widely available to the larger part of society (Jaenicke, 2013). Awareness should be created for people to know and have access to these crops as supplements to the popularly known counterparts. The various media devices available can be used to create the needed awareness.

Unavailability of a Reference Genome

The availability of a complete genetic map of BGN will increase speed breeding in this crop. As at the time of this study, the only established one is a draft genome, which was reported in the study of Chang et al. (2018b). This does not represent a full genome, as coverage is incomplete and the assembly is fragmentary. Complete coverage will give detailed knowledge of the crop which will inform breeders about various traits that can be improved for better production.

Awareness of the Right Planting Period

Finally, the BGN needs water for stabilization on the field, after which it needs little or no water. Farmers need to be wary that it does not need much water, especially once it starts podding. Because of this, planting time should be in such a way to conform with the reduction of rainfall, such that when the crop reaches the podding stage, the amount of rain would have been significantly reduced to help in the quick maturation and drying of the seeds.

IMPROVING BAMBARA GROUNDNUT PRODUCTION: A CASE FOR GENOTYPING BY SEQUENCING

Yield variation can make the effective use of BGN and other underutilized crops complicated. Thanks to advancements in the use of molecular markers in plant breeding, crops are able to produce greater yields (Zhang et al., 2017). With the aid of these markers, target loci will be located and amplified for further studies. Research to develop disease resistance, stress tolerance, and nutrient and water-use efficiency is important in crop breeding (Wani et al., 2016; Khan et al., 2020). Therefore, development of an improved BGN variety is the focus for BGN breeding. The greater the population grows, the greater the demand for food. Genomics-based innovations in NGS have expedited crop research and provided access to the previously closed frontiers of functional genomics, gene discovery, and molecular marker development in non-model plants. Through the construction of linkage maps (Meng et al., 2015), characterization of traits *via* QTL determination leading to MAS was a major achievement, causing a revolution in plant breeding, hence its translation into BGN breeding will be a success for achieving food security. Markers linked to a gene can be developed after the gene of interest has been identified through genomic studies or other applications. Similarly, the parallel sequencing of RNA (RNAseq or transcriptome profiling) is a powerful tool for transcription profiling, providing rapid access to a large collection of expressed sequences (transcriptomes). RNAseq technology has been successfully applied in several organisms, including model and non-model plants (Wang et al., 2017). It can be used as a cost effective source for developing molecular markers such as Simple Sequence Repeats (SSRs) and Small Nucleotide Polymorphisms (SNPs). It is expected that these transcriptome-derived markers will show greater transferability among closely related species than the genomic markers because of their presence in more conserved transcribed regions of the genome. These markers can also be used for comparative mapping and evolutionary studies between BGN and its closely related *Vigna* species. The complete sequencing of BGN genomes with the assistance of NGS technology will be a milestone for BGN biology and provide needed resources for its functional genomics.

A high number of molecular markers is of great importance in crop breeding. In many underutilized crops, however, the availability of sufficient molecular markers is lacking. Going forward, Diversity Array Technology (DArT) has been the "go-to" technology when it comes to developing molecular markers in underutilized crops basically because there is no need for prior sequence information. The combination of DArT with NGS resulting in DArTSeq has been used in many underutilized crops, including BGN (Ho et al., 2017; Redjeki et al., 2020) for SNP discovery.

Going forward, incorporating gene editing into BGN breeding is a possibility that is worth considering. Sequence-specific nucleases which include engineered homing endonucleases or meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the CRISPR-Cas system are used in genome editing (Gao, 2021). Protein engineering in ZFNs and TALENs is expensive, time consuming, and difficult, therefore limiting their use. These challenges have made CRISPR to be a more attractive proposition. It is faster, more precise, cheaper, and efficient. It is more versatile and has been employed in many legumes such as *Medicago truncatula* (Meng et al., 2017; Zhang H. et al., 2020), *Lotus japonica*, *Glycine max* (Zhang P. et al., 2020), and *Vigna unguiculata* (Ji et al., 2019). Removing unwanted trait elements for an improved crop is a strategy used in crop genetic improvement. This is achieved by knocking out genes of undesirable traits. Knocking out undesirable traits is the most common application of the CRISPR-Cas system (Chen K. et al., 2019). Traits such as yield, biotic, and abiotic stress resistance have been improved in crops through CRISPR-Cas application (Li R. et al., 2019; Makhotenko et al., 2019; Bouzroud et al., 2020; Zeng et al., 2020; Zheng et al., 2020; Li et al., 2021; Liu et al., 2021).

Yield is a complex trait and is controlled by factors such as grain number, grain size, grain weight, and panicle size; therefore, targeting the genes that regulate these traits through detection and gene-knock-out in BGN will improve its yield. In the study of Liu et al. (2021), for example, grain yield traits in maize were improved by editing CLE genes using the CRISPR-Cas application. Also, Gn1a and GS3 genes, which regulate grain number and grain size, respectively in rice, were targeted for yield improvement in the study of Li et al. (2016) using the CRISPR-Cas system. Therefore, translating such studies into BGN will

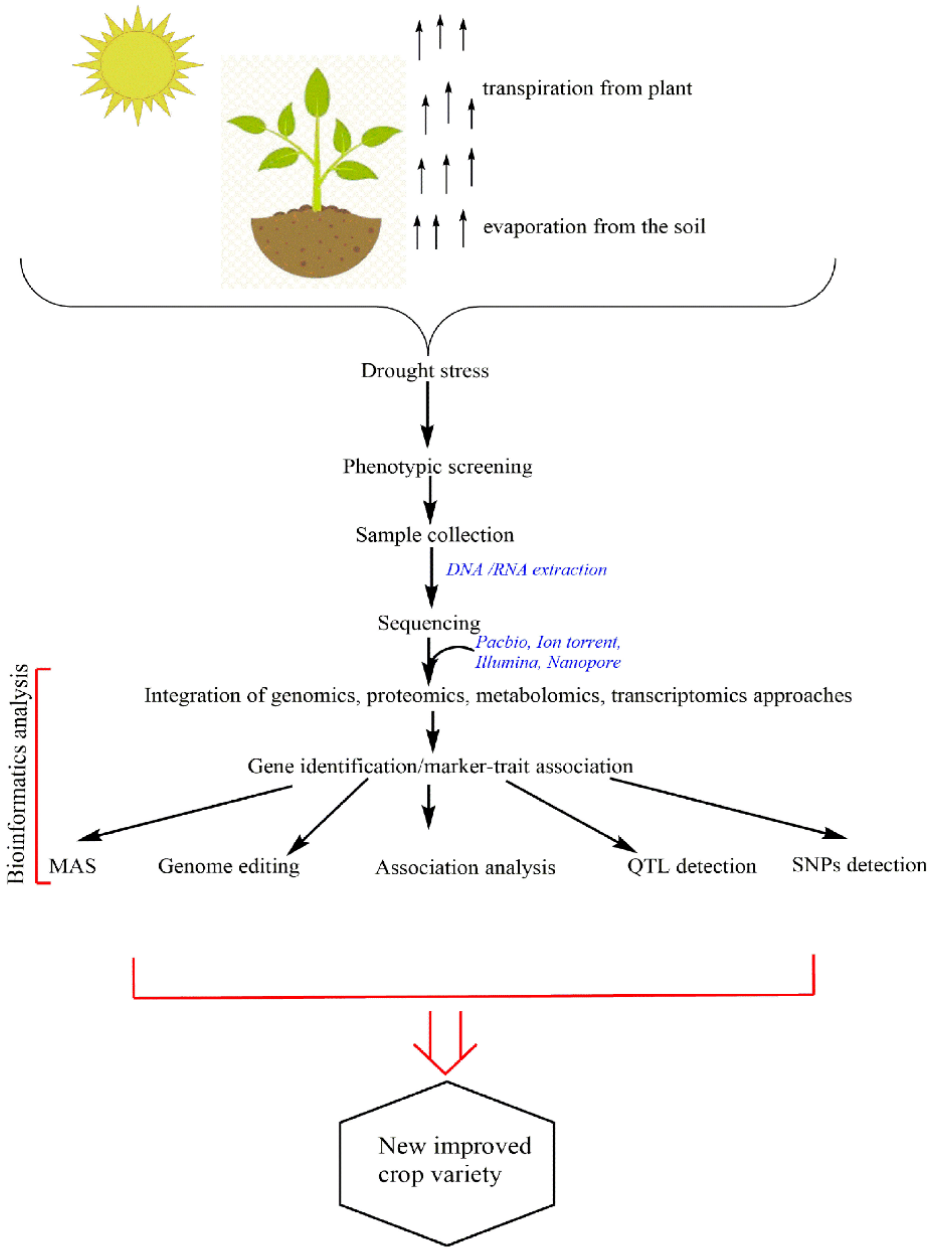

FIGURE 4 | Schematic representation of drought assessment.

provide insight into the mechanism of yield development thereby facilitating its molecular breeding for improved yield.

Bambara Groundnut is rich in nutrient compositions. The nutrients in it are sufficient that it is regarded as a complete food (Bamshaiye et al., 2011; Jideani and Diedericks, 2014). However, BGN possesses a considerate amount of antinutritional factors such as phytates, tannins, and saponins. These antinutritional factors affect protein digestibility and bioavailability of other nutrients (Halimi et al., 2019). Therefore, the removal of these antinutritional factors should be a target in BGN breeding. CRISPR-Cas technology can be applied to target genes regulating the production of these antinutritional factors. The application of CRISPR-Cas technology has been shown to improve nutrient components in seeds of soybean (Li C. et al., 2019). Abiotic stress is a complex trait controlled by many genes. CRISPR-Cas has been applied to induce and edit genomes for abiotic stress genes (Shi et al., 2017; Li R. et al., 2019). Gene-directed mutagenesis can be incorporated in BGN breeding to improve its drought tolerance capability.

Genome editing can be used to modify traits BGN to accelerate the process of domestication. However, the lack of a well-annotated genome, lack of genetic transformation methods, and suboptimal tissue regeneration protocols are bottlenecks underlying the application of gene editing tools on BGN and other orphan crops.

PROSPECTS FOR GENOMIC MARKERS IN BAMBARA GROUNDNUT BREEDING

A vast array of molecular markers has been developed in plant breeding for various purposes. The first set of markers include the Restriction Fragment Length Polymorphisms (RFLPs) which was developed in the early 1980s (Hu et al., 2020), Random Amplified Polymorphic DNA (RAPD) (Yan-Qiong et al., 2019), and Amplified Fragment Length Polymorphism (AFLP) (Yan-Qiong et al., 2019). DArT, SNP, and SSR, which have been regarded as second generation markers, have all been developed and implemented in modern plant breeding technology. Application of molecular markers are pronounced in MAS and genetic plant breeding.

Analysis of genetic diversity in crop species is based on the different phenotypic markers which are affected by the environment making it less sufficient for proper germplasm characterization. Molecular markers, on the other hand, occur at high frequencies and allow for in-depth genetic characterization. Hence combining molecular markers with phenotypic markers allow for a comprehensive characterization of germplasm collections. Even though BGN has a large genetic diversity, there are few molecular markers and genomic resources available. Meanwhile, DNA markers have been used to successfully to analyze genetic diversity in BGN germplasm collections from different parts of the world (Amadou et al., 2001; Ntundu et al., 2004; Somta et al., 2011; Olukolu et al., 2012; Fatimah and Ardiarini, 2018; Konate et al., 2019). The recently developed SNP markers enhance the effectiveness of genotyping because of their high density (Savadi et al., 2020). Therefore, this will aid large scale BGN germplasm characterization, high resolution genetic and QTL mapping.

The world's largest collection of BGN is held in GRC-IITA and various evaluation studies are being carried out (Paliwal et al., 2020). Large germplasm collection cannot be effectively characterized; therefore, a subset termed "core collections" is taken from the larger collection. The core collection can then be improved for future breeding. MAS through QTL mapping has been used to identify genes controlling important economic traits of interest in BGN and other crops. Genome-wide association studies (GWAS) is more robust and accurate in detecting QTL controlling important traits. In recent years, it has emerged as the best choice for QTL mapping in plant species (Sonah et al., 2015; Tao et al., 2020; Zhang X. et al., 2020). So far, there are no GWAS studies on BGN. However, GWAS can aid accurate detection of QTLs for important traits and their application in molecular breeding of BGN for developing improved climate-resilient varieties.

Furthermore, strategies for the development of molecular markers and their applications are based on polymorphism among individual organism genomes, hence, the nature and type of polymorphism are important. There has been full sequencing of many plant genomes such as Arabidopsis (Michael et al., 2018), rice (Du et al., 2017), soybean (Rehman et al., 2018), barley (Beier et al., 2017), maize (Jiao et al., 2017), and cowpea (Spriggs et al., 2018) but no full sequencing of BGN genome has been reported yet. The use of molecular markers for genetic diversity studies have been reported in BGN (Massawe et al., 2002, 2003; Ntundu et al., 2004; Somta et al., 2011; Olukolu et al., 2012; Siise and Massawe, 2013; Molosiwa et al., 2015; Fatimah and Ardiarini, 2018; Konate et al., 2019; Alhamdi et al., 2020).

In addition, complete pseudochromes of the BGN genome are not yet available because the sequences have been done using high-density Illumina short read data. However, plans are being made to use long read sequence data (Gregory et al., 2019). When compared to cowpea, the BGN total genome size is smaller (Lonardi et al., 2019). While Chang et al. (2018a) reported that it has higher number of protein-coding genes (31,707) than mung bean (22,427) but lower than adzuki bean (34,183), the percentage of BGN that has been functionally annotated is reportedly 98%.

BAMBARA GROUNDNUT PRODUCTION: DROUGHT AND WATER STRESS AS A CASE STUDY

Agricultural drought (AD) refers to the reduction of the water level in the soil to a point below the required amount needed by plants. AD causes an increase in soil acidity as well as affecting plant nutrient uptake. Plants need water to transport nutrients from the soil through the roots to other parts where they are needed. This decreased water level makes AD a limiting factor in the improvement of plant production. The ability of BGN to survive where other plants cannot do so has made it important for small scale farmers, especially in the developing world. The farmers benefit from the low maintenance attributes.

Both physical and molecular traits have been identified and reviewed for drought, and water stress tolerance in plants (Sahebi et al., 2018), and research focusing on these traits is the right direction for improving BGN production. The response of plants to drought is determined by the duration of water stress, the plant's developmental stage at the time of stress occurrence, and its genetic and phenotypic make-up.

Though BGN is considered a drought-tolerant crop, limited rainfall can still hamper its productivity. Drought reduces crops' resistance to pests and diseases as well as nutrient uptake. Due to inter- and intra-accession variations in genetic diversity, there will be variations in the responses of BGN accessions to drought. However, little information is available to understand the genetic basis of the drought tolerance mechanism of BGN.

Identifying the Genetic Bases for Drought Tolerance in Bambara Groundnut

A schematic representation for studying drought tolerance in BGN is shown in **Figure 4**.

The application of these techniques to BGN will help breeders in the selection for highly drought-tolerant landraces; it will help in gene location and editing to create new, improved varieties. Extensive data sets generated from sequences are analyzed using the proper bioinformatics applications and pipelines. Whole genome association studies and genome sequences for functional genomics analysis, co-expression analysis, and QTL mapping all require an advanced level of knowledge on bioinformatics because of the large data sets. System biology approaches work in synergy for a better understanding of the morphology, physiology, biochemistry, genetics, and phenotypic traits in plants (Mohanta et al., 2017) as well as their responses to various environmental challenges.

In addition, drought-tolerant genes in BGN can be identified through comparative studies with the drought-tolerant genes in other crops, and secondly through differential expressions of mRNAs in drought-stressed and non-stressed conditions. The second approach was used to identify genes expressed in stressed and non-stressed plants in BGN (Khan et al., 2017). In their study, Khan et al. (2017) identified PRR7, ATAUX2-11, CONSTANS-like 1, MYB60, AGL-83, and Zinc-finger protein genes, and concluded that these genes could serve as a basis for drought stress study in BGN. Over-expression or suppression of these genes will aid in deciphering their roles in BGN drought tolerance. Under stress conditions, plant secrete solutes like proline, polyols, abscisic acids, jasmonic acids, etc. (Ma et al., 2020). These solutes can serve as markers in BGN stress response studies.

Genome-wide association studies can be another useful technique in identifying the molecular basis of drought tolerance in BGN. Linking phenotypes to genotypes is important in deciphering the genetic basis of any trait. Drought tolerance is controlled by many traits (Parvathi et al., 2020), therefore, it will not be straight forward in regulating loci for all the associated traits. However, GWAS can be used to identify the regions of the chromosome where the loci controlling these traits are present. Identification of these regions will be a starting point for identification of drought tolerance mechanisms in less researched crops with no reference genome, like BGN.

CONCLUSION AND RECOMMENDATIONS

Modern techniques of improvement are not fully employed in orphan crops. Their crop breeding depends mostly on conventional methods such as selection and hybridization. However, limited numbers of breeders implement modern techniques such as marker-assisted breeding and transgenics. Genomic information such as the whole genome sequence has not gathered pace for any orphan crop. To feed the ever-increasing population in Africa, an agricultural revolution is needed to boost the productivity of orphan crops using modern technologies that have proven to be effective for the major crops of the world.

The application of NGS technology is greatly increasing our knowledge of plant genomics. This has been established in some plants where functional genomics would never have been a reality with earlier sequencing technologies. NGS will enable the quest for higher quality BGN reference genomes and resequencing of many cultivated species genotypes and wild relative accessions soon. With the availability of these rich genetic resources and high-quality genotyping platforms, the functional genomics studies of BGN are entering a new era.

A comprehensive survey of genetic diversity in BGN landraces and wild relatives will deepen our understanding of the genetic basis underlying the domestication and evolution of this orphan crop. Using the NGS technology and informatics combined with information on genetic variation will provide the push needed for BGN to catch up with other crops in studies on functional genomics. The time for genomics-assisted BGN breeding is finally here.

AUTHOR CONTRIBUTIONS

OSO researched the data, wrote, and edited the manuscript. OO, OOB, and MA reviewed and supervised the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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CHAPTER 3: ARTICLE 2

Genetic diversity and environmental influence on growth and yield parameters of Bambara groundnut

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Genetic Diversity and Environmental Influence on Growth and Yield Parameters of Bambara Groundnut

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Bambara groundnut (BGN) is a drought-tolerant crop majorly cultivated in sub-Saharan Africa. Due to a lack of extensive research, marginalization, lack of awareness, and lack of available fund among others, this crop's improvement has been limited. The development of this crop depends on evaluation and selection of unique and stable breeding lines in different environments. This study aims to estimate genetic diversity using morphological traits at different locations in 95 accessions of BGN collected from the Genebank of the International Institute of Tropical Agriculture (IITA), Ibadan. The experiment was carried out in three replicates at IITA experimental sites in two agroecological zones in Ibadan (7°40′19.62″ N, 3°91′73.13″ E) and Ikenne (6°51′00.873″ N, 3°41′48.528″ E) using a randomized complete block design. Ten vegetative growth traits and eight yield traits were scored. The data was subjected to ANOVA, PCA, correlation, and cluster analysis. Estimations of genetic parameters and broad sense heritability were carried out on the traits. ANOVA revealed significant variation in each trait except for days to emergence. Significant variation was also observed for accession and location interactions (genotype x environment interactions) for plant height, leaf length, leaf width, chlorophyll content, number of petioles, germination count, number of pods, number of seeds, seed length, seed width, and yield. PC1 and PC2 show 42.3% of the total variations observed by the PC, with seed thickness contributing more to PC1 and the number of seeds contributing more to PC2. Cluster analysis categorized the accessions into four distinct groups. The number of pods had the highest genotypic coefficient of variation of 32.55% and the phenotypic coefficient of variation of 97.61%, while seed length (0.63), seed width (0.54), and seed thickness (0.58) have high heritability values. The genetic advance was highest in yield (76.15%) and lowest in days to 50% germination (0.21%). This study can be used to predict appropriate agroecological zones for the planting of BGN while the knowledge of the diversity of the accessions based on the traits could serve a guide in selecting the best trait for the improvement of the crop.

Keywords: Bambara groundnut, genetic diversity, genotype by environment interaction, heritability, multivariate analysis, underutilized legumes, food security

INTRODUCTION

Bambara groundnut (BGN) [*Vigna subterranea* (L) Verdc.] is a leguminous species that produces edible seeds. Human populations in the tropics include BGN seeds in their daily diet to compensate for the lack of proteins in their foods. Compared to other legumes, there is limited information about the extent of variation in BGN accessions (Zenabou et al., 2016). BGN represents the 3rd most important grain legume in tropical Africa. It is characterized by resistance to drought, high temperatures, and high nutrient composition. It is an indigenous African crop that has been cultivated since the 17th century (Laurette et al., 2015). It has many local names based on the area of cultivation such as jugo beans (South Africa), epa roro (Yoruba tribe in Nigeria), ntoyo ciBemba (Zambia), etc. (Olufemi, 2019). The crop is highly nutritious (Halimi et al., 2019) and grown in different countries in and out of Africa. Despite a lot of promise held from the reports of various studies (Obidiebube et al., 2020; Khan et al., 2021; Olanrewaju et al., 2021), BGN is still an underutilized crop. As a drought-tolerant legume (Mayes et al., 2019), it deserves greater attention than it is presently receiving. With the challenges of climate change affecting food production and a limited number of crops being cultivated, research into BGN should be intensified to improve the crop. Improvement of this crop will greatly impact and help in addressing the problem of food insecurity in Africa and beyond.

Most of the named cultivars have emerged majorly from the seed collection locations (Cook, 2017). It has both prostrate and erect forms and grows to a height of 0.30–0.35 m. Like the common groundnut, it has compound leaves of three leaflets which are either bunched type or spreading. The former type is self-pollinating while the latter are cross-pollinating. The branched stems of the plant root at the nodes to form a bunched herbaceous annual with a thick taproot which forms a profusion of lateral roots toward its tip (Mubaiwa et al., 2018; Valombola et al., 2019). It is an autogamous plant having pale-yellow flowers on the branching stems (Molosiwa et al., 2015; Aliyu et al., 2016). After fertilization, the stem grows into the soil with the already developed seed. The seed pod which is about 1.25–2.5 cm in diameter ends up about 1 cm beneath the soil surface having one or two seeds formed. The seeds are mostly formed after 40 days of fertilization.

The yield of any crop is a major cause for concern, especially in this era when climate change and population increase are creating challenges to sustainable food production. In finding solution to this problem, many studies have been carried out with the aim of yield improvement (Bailey-Serres et al., 2019; Oldfield et al., 2019; Schauberger et al., 2019). BGN is one of the many underutilized crops that tend to fully complement and stand at par with the major crop counterparts in terms nutrition contents (Atoyebi et al., 2017b). Hence, its importance in achieving food security. However, to fully harness its potential, good understanding of yield and yield components is needed in building an efficient breeding program for the crop.

Multi-environment trials (MET) are key for selection and recommendation (Kumar et al., 2019). MET aid in selection in terms of varieties with best yield observed in the environments under study. Hence, recommendations can be made for the most suitable environment. BGN yield is affected by soil properties and environmental factors such as rainfall and temperature. Climate change impact has not helped the production of crops globally, including BGN, thereby hampering food security (Mayes et al., 2019). Genotype x environment interactions (GEI) lead to different responses from various plant accessions for each trait. This plays a vital role in selection processes. Most farmers also grew landraces that segregated, resulting in seasonal yield variability, and lacked farmer-preferred traits like high palatability and quick processing (Mabhaudhi et al., 2013). Thus, new and improved cultivars are required to meet market demands and environmental constraints. So, improving Bambara productivity is required to justify its inclusion in diverse cropping systems. Understanding Bambara's morphological and physiological traits is the first step to efficiently exploiting its genetic diversity. This method will also help breeders and farmers by providing vital baseline data on known lines. It will also help characterize useful germplasm for breeding programs and improve selection efficiency for environmental conditions. Therefore, METs are essential in selecting high yielding accessions of crop varieties suitable for specific environments although, the adaptation of specific genotypes to various environments can be gene-specific (Olanrewaju et al., 2021). In addition, most agromorphological studies on BGN have focused on only one location with the only exception being the study by Mogale (2018).

With a view of improving BGN production, this study looks at the agromorphological variations in the various accessions reported as affected by two geographical zones in southwest Nigeria. Since these accessions originated from various sources, this study also looks at the responses in the vegetative and yield traits of these accessions based on their origin using the principal component analysis. The outcome of this study could be used to make effective strategies for the improvement of BGN for food and nutrition security.

MATERIALS AND METHODS

Study Site Description

The study was conducted in two different agroecological zones: Ibadan (7°40′19.62″ N, 3°91′73.13″ E), which is a derived savannah, and Ikenne (6°51′00.873″ N, 3°41′48.528″ E), which is a rain forest. International Institute of Tropical Agriculture (IITA) field stations in Ibadan and Ikenne were used. The study was carried out in the 2018 and 2019 planting seasons. The average climate data for the study sites are shown in **Table 1**.

Soil Sampling and Analysis

Topsoil samples were collected randomly from 0 to 15 cm across the plot area using the soil auger and these were bulked together to obtain a composite sample for analysis before establishing the experiment. The soil sample was dried under shade, passed through 2 mm sieve for subsequent chemical analyses [sand, clay, silt, pH, organic carbon (OC), total N, exchangeable Ca, Mg, K, available P, Na, Mn, Cu, Fe, and Zn] and particle size distribution at the onset of the experiment.

TABLE 1 | Monthly mean meteorological data of the experimental sites during Bambara groundnut (BGN) growing season (average of 2018–2019 and 2019–2020 crop season).

			August	September	October	November	December	January
Ibadan	2019/2020 2018/2019	Average temperature (°C)	25	25	25	30	30	29
		Average precipitation (mm)	94.8	99.2	53.5	3.3	0	37.6
		Average relative humidity (%)	85	87	86	69	53	62
	2019/2020 2018/2019	Average temperature (°C)	26	26	26	28	29	29
		Average precipitation (mm)	266.7	319.8	661	69.8	1.3	0.9
		Average relative humidity (%)	82	83	85	77	62	46
Ikenne	2019/2020 2018/2019	Average temperature (°C)	26	26	27	28	29	29
		Average precipitation (mm)	113.2	163.9	69.3	13.3	0.6	45.1
		Average relative humidity (%)	87	90	87	86	79	82
	2019/2020 2018/2019	Average temperature (°C)	26	26	26	28	28	28
		Average precipitation (mm)	390.3	300.9	565.8	145.4	12.7	1
		Average relative humidity (%)	85	88	89	85	83	71

Plant Materials

A set of 95 accessions of BGN that have not been previously characterized were collected from the BGN germplasm being conserved at the Genetic Resources Center, IITA, Ibadan. The accessions originated from western to southern Africa. Out of the total number of accessions used, the origins of 34 of them were not available hence they are reported as unknown (**Figure 1**).

Field Trials and Phenotyping

Randomized complete block design was used in the field trials. Sixty seeds of each accession were planted during the 2018 and 2019 planting seasons. The accessions were planted in three replicates with each replicate having 20 plants per accession on a plot which were later thinned to 10 plants at 2 weeks after emergence. The length of each plot was 3 m with 0.3 m spacing between each plant and a row spacing of 0.7 m between each plot. Each replicate contains 3 blocks which were separated by 1 m spacing and the replicates were separated from one another by 2 m spacing. The first planting in 2018 was on the 1st and 12th of September while that of 2019 season was on the 26th of August and 16th of September in Ikenne and Ibadan, respectively. Plants were rainfed until the stop of rain before irrigation was applied once in a week. According to the BGN descriptor (Ipgri/Iita/Bamnet, 2000), 10 quantitative vegetative traits were scored (**Table 2**).

The number of pods, hundred seed weight (g), and total seed weight (g) were determined immediately after drying of the pods. The characteristics of the seeds were determined according to the method described by Saka et al. (2005). For each seed, individual seed length (L), width (W), and thickness (T) were measured using an electronic vernier caliper. T was defined as the distance from the seed's eye to the opposite end, while L and W taking in the two opposite perpendicular directions of eye seed represented the major and the minor seed diameters. Seeds were weighed to obtain a yield of the grain, which was then converted to hectare using the formula:

$$\text{Yield}\left(\frac{\text{kg}}{\text{ha}}\right) = \frac{\text{plot yield}^{*}10000}{\text{plot area}}$$

For each accession, a triplicate of one hundred seed weight was obtained using an electronic balance.

Statistical Analysis

Data were checked for normality using the Shapiro-Wilk test before further analysis and all analyses were done using the R statistical package (R Core Team, 2019). The pastecs package was used to compare the responses of each trait at the various years and locations. The different traits were submitted to an ANOVA by fitting the model with the lmer function of the lme4 package in R, with all sources of variations set as random effects. Locations, blocks, replicates, accessions, and year of planting were taken as factors. The interaction effect between the genotype, year, and locations was accounted for with all sources of variations considered as random effects. Fischer's least significant difference (F-LSD) at a probability level of 5% was used to separate the means that were significantly different. PCA was done using the FactoMineR package (Lê et al., 2008) and Pearson correlation was performed using the corr. function of the stats package in R. A hierarchical cluster analysis was performed using the ward D2 method in a cluster R package (Maechler et al., 2019).

The variability was estimated using the variability package as per procedure for analysis of variance suggested by Panse and Sukhatme (1967), phenotypic coefficient variation (PCV) and genotypic coefficient variation (GCV) were calculated by the formula by Burton (1952) heritability in broad sense (h^2) by Burton and Devane (1952) and genetic advance i.e., the expected genetic gain were calculated by using the procedure given by Johnson et al. (1955). The estimated values of PCV and GCV were categorized as described by Khan et al. (2020), 0–10% for low, 10–20% for intermediate, and $\geq$ 20% for high. Heritability was classified as 0–30% for low, 30–60% for intermediate, and >60% as high (Johnson et al., 1955). Genetic advance as percentage of the mean was classified as follows: 0–10% as Low, 10–20% as Moderate, and 20% and above as High. The value of k was taken as 2.06 assuming 5% selection intensity following the method of Adewale et al. (2010).

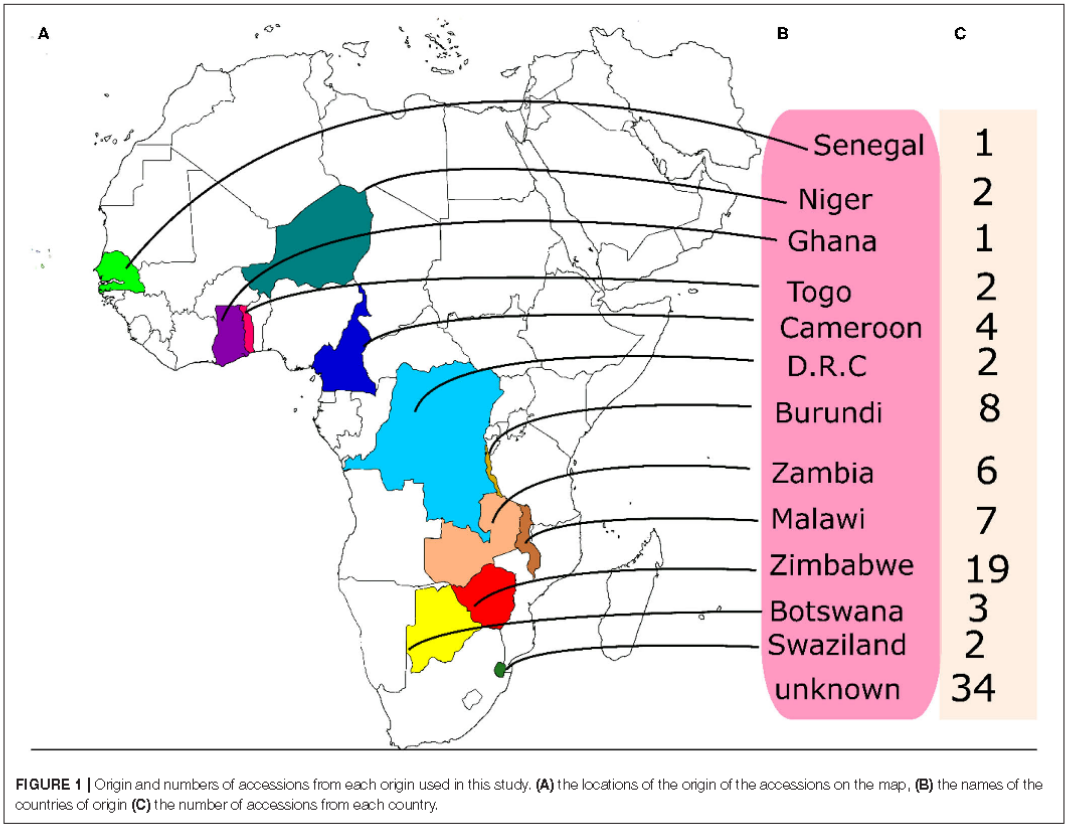

FIGURE 1 | Origin and numbers of accessions from each origin used in this study. **(A)** the locations of the origin of the accessions on the map, **(B)** the names of the countries of origin **(C)** the number of accessions from each country.

RESULTS

Soil Analysis

Soil physicochemical properties for the two locations at both seasons are represented in **Table 3**. High amount of sand, calcium, magnesium, potassium, sodium, manganese, iron, cupper, and zinc were recorded in Ikenne compared to Ibadan for the 2018 planting season while in 2019 planting season, Ibadan had the highest pH, sand, nitrogen, organic carbon, manganese, iron, and zinc when compared with Ikenne (**Table 3**). Crop yield has been shown to be influenced by soil and climatic conditions. Crops respond differently to various soil types (Tolk et al., 1999). BGN produces well in sandy soils. Even though sandy soil inhibits crop emergence, BGN benefits from it because they bear fruit underground. Sandy soil has a porous structure with large pores, allowing pods to grow. When sandy soils dry out, they produce thin, loose fissures (Tester, 1990). This is an advantageous trait, especially in the semi-arid tropics where rainfall is unpredictable and soil is subjected to prolonged periods of dryness. Although clay soil has a high-water retention capacity, it expands when wet and contracts when dry over long periods of time (Brady and Weil, 2010).

Traits Analysis

The number of petioles (20–280), chlorophyll content (14.2–99.2), hundred seed weight (0–380), and total seed weight (0–771.5) were among the traits with the largest range of values (**Table 4**). There was high variation among accessions for yield and yield characters, with the highest coefficient of variation (CV) observed in number of pods (93%), number of seeds (86%), and total seed weight (66%), followed by yield (39%) and hundred seed weight (36%), both showing CV <50%. These were followed by chlorophyll content (29%), leaf width (26%), days to emergence (25%), plant height (24%), petiole length (23%), and days to 50% germination (21%). The remaining traits showed CV <20%.

Analysis of variance (ANOVA) showed that location was highly significant for all the traits ($p < 0.0001$) except for plant height ($p < 0.001$), leaf length (not significant), total seed weight ($p < 0.05$), and yield ($p < 0.01$) (**Table 5**). A significant block effect was observed for all traits except hundred seed weight. The accessions varied significantly in plant height, leaf length and width, days to flowering, chlorophyll content, number of petioles, germination count, petiole length, number of pods

TABLE 2 | Traits scored and their abbreviations.

Traits scored	Abbreviations	Description and time scored
Days to emergence	DTE	Number of days from sowing to seedling emergence
Germination Count	GCT	Number of successfully established plants taken at 2 weeks after sowing
Days to 50% germination	DT50G	Number of days from sowing to when half of the seeds germinate
chlorophyll content	CHLCON	Amount of chlorophyll measured with SPAD meter before sunrise on five healthy plants at 12 weeks
Days to 1st Flower	DTF	Number of days from sowing to first flowering
Number of petioles/Accession	NPET	Recorded 12 weeks after planting; average count of petioles of five healthy plants.
Petiole length/Accession	PEL (cm)	Recorded 12 weeks after planting; average length of three leaves at the fourth node of five healthy plants
Plant height/Accession	PH (cm)	Measured from the ground level (at the base of the plant) to the tip of the highest point, including the terminal leaflet. Recorded 12 weeks after planting; average height of five plants
Leaf length/Accession	LLE (cm)	Recorded 12 weeks after planting; average length of three leaves at the fourth node of five healthy plants
Leaf width/Accession	LWI (cm)	Recorded 12 weeks after planting; average width of three leaves at the fourth node of five healthy plants
Number of pods/Accession	NPOD	Average number of 10 plants
Number of seeds/Accession	NSEED	Average number of 10 pods recorded within 2 months after harvest
Hundred seed weight	HSWT (g)	Recorded within 2 months after harvest (at 12% moisture content)
Total seeds weight/Accession	TSWT (g)	Recorded within 2 months after harvest (at 12% moisture content)
Seed length	SEEDL (cm)	Recorded within 2 months after harvest; average length of 10 seeds
Seed width	SEEDW (cm)	Recorded within 2 months after harvest; average width of 10 seeds
Seed thickness	SEEDT (cm)	Recorded within 2 months after harvest; average thickness of 10 seeds
Yield/Accession	(kg/ha)	Weight of dried seed (at 12% moisture content)

TABLE 3 | Soil properties at the beginning of the experiment for individual locations and seasons.

Properties	2018		2019	
	Ibadan	Ikenne	Ibadan	Ikenne
Sand%	73.67	80.33	79.33	75.00
Clay%	19.67	13.67	14.00	15.67
Silt%	6.67	6.00	6.67	9.33
pH	6.70	6.42	6.59	5.02
%N	0.17	0.10	0.10	0.09
Bray P	13.45	22.48	11.27	18.89
%OC	1.02	0.41	0.44	0.41
Ca (cmol/kg)	1.13	3.53	1.19	3.53
Mg (cmol/kg)	0.07	0.80	0.27	0.80
K (cmol/kg)	0.14	0.56	0.22	0.56
Na (cmol/kg)	0.06	0.08	0.05	0.08
Mn (ppm)	150.39	154.82	135.30	112.15
Fe (ppm)	86.22	85.84	89.46	85.58
Cu (ppm)	0.55	1.17	0.20	1.17
Zn (ppm)	1.05	1.96	2.72	1.96

per plant, number of seeds, hundred seed weight, total seed weight, seed length, seed width, seed thickness, and yield ($p < 0.0001$) while days to 50% germination ($p < 0.001$) and days to emergence ($p < 0.01$) were also significant. Remarkably, location and accession interaction effect were highly significant on plant height, chlorophyll content, number of petioles, germination count, number of pods, and number of seeds ($p < 0.0001$), while leaflength, leaf width, and yield were significant at $p < 0.001$, days to 50% germination and seed length were significant at $p < 0.01$ and at $p < 0.05$, days to emergence, petiole length, and seed width were significant. Furthermore, interaction between accession and year was highly significant for plant height, number of pods, and number of seeds ($p < 0.0001$), leaf length ($p < 0.001$), and leaf width ($p < 0.01$). There was a high significant effect of location, accession, and year interaction on leaf length, leaf width, petiole length, number of pods and seeds ($p < 0.0001$), plant height, and days to flowering ($p < 0.01$). The mean comparison from the LSD analysis for each accession, location, and the year is shown in **Supplementary Table S1**. The result in **Supplementary Table S1** showed that high level of variability and heterogeneity exist among accessions, locations, and years in response to the traits scored.

Principal Component Analysis

The principal component of the variances taken by each accession and the overall component response of the accessions on the traits over the environments were represented in a biplot (**Figure 2**). PC1 (Dim1) and PC2 (Dim2) accounted for 42.3% of the total variances observed. PC1 accounted for 24.67% of the total variations while PC2 accounted for 17.63%. PCA biplot loading both variables and accessions showed how strongly each trait influences a PC and how they are correlated to each other. The lesser angle between two vectors (**Supplementary Figure S6**)

TABLE 4 | Summary statistics of the traits scored.

	Min	Max	Range	Mean	StdError	StdDev	CV
DTE	3	13	10	7.63	0.06	1.88	0.25
DT50G	6	18	12	11.68	0.07	2.43	0.21
GCT	2	10	8	8.2	0.04	1.46	0.18
CHLCON	14.2	99.2	85	39.78	0.34	11.47	0.29
DTF	28	49	21	38.11	0.14	4.62	0.12
PH	7.3	48.1	40.8	25.37	0.18	6.15	0.24
LLE	3.3	12.5	9.2	6.45	0.04	1.22	0.19
LWI	0.6	7.4	6.8	2.78	0.02	0.72	0.26
PEL	2.5	33.8	31.3	16.29	0.11	3.79	0.23
NPET	20	280	260	95.74	1.25	42.13	0.44
NPOD	0	1,133	1,133	192.68	5.33	179.93	0.93
NSEED	0	1,062	1,062	184.24	4.69	158.34	0.86
HSWT	0	380	380	75.47	0.8	27.12	0.36
TSWT	0	771.5	771.5	153.51	3.01	101.7	0.66
SEEDL	2.4	17.81	15.41	11.55	0.05	1.53	0.13
SEEDW	0.34	14.58	14.24	9.17	0.03	1.14	0.12
SEEDT	0.8	14.78	13.98	9.5	0.04	1.26	0.13
YIELD	0	1266.67	1266.67	317.12	3.7	124.97	0.39

*Each trait with its mean, maximum, range, standard error, standard deviation, coefficient of variation, and minimum values shown. Min, Minimum; Max, Maximum; StdError, Standard Error; StdDev, Standard Deviation; CV, coefficient of Variation. *n = 3.*

indicated higher and positive correlation (e.g., NPOD and NSEED, SEEDW, and SEEDT). However, when angle between two vectors form 90°, it indicated no correlation and when it is more than 90°-180°, it indicated negative correlation between the traits (e.g., DTF and PH). Among the variables, seed width (19.53%), seed thickness (19.58%), hundred seed weight (16.98%), seed length (15.93%), and yield (9.76%) were the major contributing traits in PC1 while number of seeds (21.78%), number of pods (18.48%), total seed weight (13.96%), plant height (9.12%), and petiole length (8.93%) had the highest contributions to PC2 (**Table 6**; **Supplementary Figures S3–S7**). The contributions of each trait to all components were represented in **Supplementary Figures S1–S3**. The individual PCA plot (**Supplementary Figure S7**) showed that most of the accessions were dispersed at low distances while few were dispersed at high distances as reflected by eigenvector (**Table 6**). From the biplot, we can conclude that the accessions loading on PC1 were high yielding with thick and long seeds while at the same time having high hundred seed weight. Accessions loading on PC2 have high number of seeds, number of pods, and total seed weight. Furthermore, these accessions on PC2 were tall plants with longer petioles (**Figure 2**).

Cluster Analysis

The accessions were grouped into 4 groups based on the agromorphological traits (**Figure 3**), with the clusters in red having the highest number of accessions (37 accessions) followed by the ones in green (30), blue had 11, and the purple cluster with 17 accessions. The clusters in red consisted of TVSu-2017, TVSu-1557, TVSu-2046, TVSu-2056, TVSu-1470, TVSu-1956, TVSu-2043, TVSu-1701, TVSu-2031, TVSu-2071, TVSu-2068, TVSu-2074, TVSu-2042, TVSu-2076, TVSu-1921, TVSu-1905, TVSu-2038, TVSu-2065, TVSu-2085, TVSu-1920, TVSu-2060, TVSu-1765, TVSu-2000, TVSu-2055, TVSu-1915, TVSu-1918, TVSu-2067, TVSu-1680, TVSu-1962, TVSu-2034, TVSu-1912, TVSu-2045, TVSu-1745, TVSu-2075, TVSu-1785, TVSu-1739, and TVSu-1959 while the green cluster is made up of TVSu-1763, TVSu-1951, TVSu-1547, TVSu-1742, TVSu-1892, TVSu-1943, TVSu-1787, TVSu-1589, TVSu-1758, TVSu-1945, TVSu-1879, TVSu-2051, TVSu-1733, TVSu-1937, TVSu-1863, TVSu-1972, TVSu-1874, TVSu-1740, TVSu-1941, TVSu-2083, TVSu-1895, TVSu-2032, TVSu-1850, TVSu-1899, TVSu-1724, TVSu-1851, TVSu-1859, TVSu-1939, TVSu-1823, and TVSu-1868, and the blue cluster consisted of TVSu-2019, TVSu-1930, TVSu-2003, TVSu-2018, TVSu-2022, TVSu-1964, TVSu-2025, TVSu-2030, TVSu-1957, TVSu-2020, and TVSu-2021. Finally, the purple cluster consisted of TVSu-1866, TVSu-1764, TVSu-1898, TVSu-1649, TVSu-1952, TVSu-1538, TVSu-1664, TVSu-1979, TVSu-2048, TVSu-1706, TVSu-1839, TVSu-1574, TVSu-2086, TVSu-1663, TVSu-1836, TVSu-1843, and TVSu-1923 (**Figure 3**).

Accessions in the red cluster were characterized by high mean values for days to emergence, plant height, seed thickness, seed width, seed length, hundred seed weight, and yield while those in the green cluster were characterized by high mean values for number of petioles, number of pods, number of seeds, and total seed weight. On the other hand, the blue and purple clusters were both characterized by accessions with low leaf width, plant height, number of pods, number of seeds, hundred seed weight, and total seed weight. Interestingly, the red cluster is dominated with accessions whose origins are not known (**Supplementary Table S4**). We can say that these accessions might be from the same origin or region.

TABLE 5 | ANOVA result for traits scored.

SOV	Df	PH	LLE	LWI	DTF	DTE	CHL	NPET	GCT	PEL	DT50G	NPOD	NSEED	HSWT	TSWT	SL	SW	ST	YLD
Lctns(L)	1	120.9**	1.04^{ns}	7.27***	8702.0***	342.04***	18556.6***	189,875***	264.0***	498.33***	1709.1***	549,782***	1,215,454***	20,048***	22,326	135.59***	40.76***	72.68***	77,311*
Block	4	1019.5***	26.16***	7.22***	147.0***	9.74*	212.5	17,465***	7.26***	194.87***	16.98**	1,003,097***	56,0362***	812^{ns}	224,046***	4.53*	5.94***	3.02*	57,470**
Rep	2	0.5	1.70	0.65	23.4	168.94	2697.9	3,033	7.50	42.02	57.36	12,005	4,413	355	8,930	0.67	0.21	0.94	27,826
Acons(A)	94	83.0***	2.12***	1.14***	6.4***	4.17*	239.9***	3515***	3.75***	26.22***	6.63**	50,848***	35,141***	1,421***	11,576***	9.96***	4.56***	6.09***	28,482***
Year(Y)	1	9222.4***	187.91***	33.10***	2528.8***	0.55^{ns}	87.0^{ns}	113,973***	180.67***	1172.03***	4.01^{ns}	4,597,305***	3,773,330,440***	47,969***	876,694***	13.84**	40.85***	7.14**	96,718**
L*A	94	23.1***	1.27**	0.48**	4.5^{ns}	3.74	215.1***	2,231***	4.14***	10.21	5.76*	23,977***	18,538***	490^{ns}	6,030^{ns}	1.82*	1.04	1.10^{ns}	21,245**
L*Y	1	6677.8***	165.09***	39.27***	7490.2***	0.50 ^{ns}	81.0^{ns}	108,052***	166.88***	1192.74***	0.03^{ns}	8,077,471***	5,127,831***	16,767***	1,963,188***	43.27***	34.13***	67.07***	6532^{ns}
A*Y	94	23.2***	1.30**	0.41*	4.2^{ns}	1.59 ^{ns}	21.07^{ns}	682^{ns}	0.62^{ns}	9.90^{ns}	1.82^{ns}	28,598***	24,255***	536^{ns}	7234^{ns}	1.48^{ns}	1.02^{ns}	1.18^{ns}	8,855^{ns}
L*A*Y	94	18.5**	1.58***	0.52***	5.8**	1.63^{ns}	21.6^{ns}	695^{ns}	0.52^{ns}	20.96***	1.82^{ns}	22,755***	19,397***	690^{ns}	7230^{ns}	1.28^{ns}	0.8^{ns}	1.05^{ns}	14,147^{ns}
Res	754	12.0	0.87	0.32	4.0	3.0	103.7	1,146	1.23	8.42	4.441	10,284	9,927	602	6,617	1.42	0.85	1.0	13,909
GM		25.39	0.82	0.44	1.58	0.87	1.6	1.94	0.9	1.23	1.06	6.81	6.89	6.21	6.78	3.52	3.18	3.23	8.26
LSD		2.78	0.75	0.45	1.60	1.39	8.16	27.13	0.89	2.32	1.68	81.28	79.85	19.67	65.19	0.95	0.74	0.80	94.52

Trait values indicate the mean square values; Lctns, locations; Acons, accessions; Res, residuals; GM, grandmean; Df, degree of freedom; CHL, chlorophyll content; SL, seed length; SW, seed width; ST, seed thickness; YLD, yield; other traits are as represented in ***Table 2****.*

****Highly significant at p < 0.0001.*

***Highly significant at p < 0.001.*

**Highly significant at p < 0.01.*

. Significant at p <0.05.

ns, no significance.

Other accessions present in this cluster are from Burundi (1), Cameroon (3), DRC (1), Ghana (1), Togo (2), Malawi (2), Zambia (2), and Zimbabwe (3). Remarkably, the green cluster consist of accessions from the southern region of Africa which include Botswana, Malawi, Swaziland, Zambia, and Zimbabwe. Furthermore, accessions from Zimbabwe are more dominant in this cluster. Like the green cluster, the blue cluster is also dominated by accessions from southern Africa with Burundi having the highest number of accessions in this cluster. Finally, the purple cluster largely consists of accessions from the western part of Africa with Senegal producing the highest number of accessions (**Supplementary Table S4**). Hence, accessions from the red clusters which are majorly from unknown origin are more desirable for selection for improved breeding programs while those in the green cluster, dominated by accessions from southern Africa, can also be considered.

Correlation Analysis of the Traits

There were lots of significant correlations among the traits scored (**Figure 4**). The level of significance of the correlations at $p < 0.05$ is shown by the asterisks. Either positive or negative correlations, the asterisks indicate if it is statistically significant or not. The red colors showed negative correlations while the blue colors showed positive correlations. The deeper the colors, the stronger the correlations. The correlation matrix showed that plant height had a positive correlation with leaf length ($r = 0.51$, $p < 0.05$), leaf width ($r = 0.67$, $p < 0.01$), germination count ($r = 0.09$, $p = 0.72$), days to flowering ($r = 0.09$, $p = 0.73$), number of petioles ($r = 0.12$, $p = 0.64$), petiole length ($r = 0.92$, $p < 0.001$), number of pods (r = 0.07, $p = 0.78$), number of seeds ($r = 0.17$, $p = 0.5$), hundred seed weight ($r = 0.09$, $p = 0.72$), total seed weight ($r = 0.24$, $p = 0.34$), seed length ($r = 0.19$, $p = 0.44$), seed width ($r = 0.12$, $p = 0.63$), seed thickness ($r = 0.16$, $p = 0.52$), and yield ($r = 0.00$, $p = 0.96$) but negatively correlates with the remaining traits (**Supplementary Tables S2, S3**). Among the positive correlations however, correlations with leaf length, leaf width, petiole length, and chlorophyll content were not significant (**Supplementary Tables S2, S3**). Leaf length, in addition to plant height, positively correlated with leaf width ($r = 0.43$, $p = 0.07$), germination count ($r = 0.14$, $p = 0.59$), days to flowering ($r = 0.29$, $p = 0.24$), petiole length ($r = 0.57$, $p < 0.01$), number of pods ($r = 0.16$, $p = 0.52$), and number of seeds ($r = 0.23$, $p = 0.35$) but correlation with petiole length is not significant (**Supplementary Tables S2, S3**). Furthermore, leaf width correlated positively with germination count ($r = 0.28$, $p = 0.27$), days to flowering ($r = 0.22$, $p = 0.38$), petiole length ($r = 0.59$, $p < 0.01$), hundred seed weight ($r = 0.25$, $p = 0.32$), total seed weight ($r = 0.02$, $p = 0.95$), seed length ($r = 0.50$, $p < 0.05$), seed width ($r = 0.42$, $p = 0.08$), seed thickness ($r = 0.44$, $p = 0.07$), and yield ($r = 0.06$, $p = 0.82$), while days to emergence also had a positive correlation with days to 50% germination ($r = 0.94$, $p < 0.001$), chlorophyll content ($r = 0.40$, $p < 0.5$), and days to flowering ($r = 0.40$, $p < 0.5$). Days to 50% germination, on the other hand, correlated positively with chlorophyll content ($r = 0.43$, $p = 0.07$), days to flowering ($r = 0.29$, $p = 0.24$), and yield ($r = 0.02$, $p = 0.92$) while chlorophyll content correlated positively with days to flowering and yield (**Supplementary Tables S2, S3**).

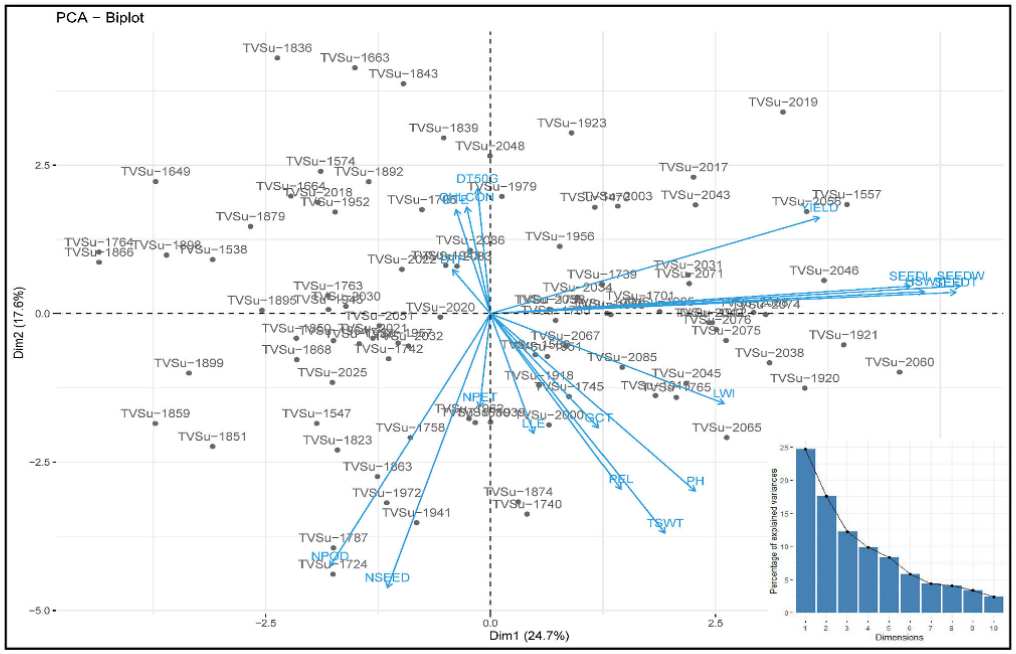

FIGURE 2 | PC 1 and PC 2 biplot using quantitative trait scores of the Bambara groundnut (BGN) accessions. Each accession is represented according to its origin. Inset is the scree plot showing the explained variances.

In addition, germination count had a positive correlation with petiole length ($r = 0.03$, $p = 0.89$), number of pods ($r = 0.18$, $p < 0.5$), number of seeds ($r = 0.22$, $p < 0.5$), hundred seed weight ($r = 0.19$, $p < 0.5$), total seed weight ($r = 0.46$, $p \leq 0.05$), seed length ($r = 0.19$, $p < 0.5$), seed width($r = 0.28$, $p < 0.5$), and seed thickness ($r = 0.29$, $p < 0.5$), while days to flowering correlated positively with petiole length ($r = 0.25$, $p < 0.5$). Number of petioles had a positive correlation with petiole length ($r = 0.06$, $p = 0.8$), number of pods ($r = 0.43$, $p = 0.07$), number of seeds ($r = 0.39$, $p = 0.11$), total seed weight ($r = 0.31$, $p = 0.21$), and yield ($r = 0.05$, $p = 0.85$) while petiole length had positive correlation with number of pods ($r = 0.17$, $p = 0.51$), number of seeds ($r = 0.23$, $p < 0.5$), and total seed weight ($r = 0.17$, $p < 0.5$). Number of pods positively correlated with number of seeds ($r = 1.0$, $p < 0.001$) and total seed weight ($r = 0.62$, $p < 0.01$); number of seeds positively correlated with number of petioles ($r = 0.39$, $p < 0.5$) and total seed weight ($r = 0.67$, $p < 0.01$). Hundred seed weight had positive correlations with total seed weight ($r = 0.23$, $p < 0.5$), seed length ($r = 0.88$, $p < 0.001$), seed width ($r = 0.95$, $p < 0.001$), seed thickness ($r = 0.94$, $p < 0.001$), and yield ($r = 0.86$, $p < 0.001$); total seed weight had a positive correlation with seed length ($r = 0.03$, $p = 0.89$), seed width ($r = 0.14$, $p = 0.59$), seed thickness ($r = 0.16$, $p = 0.53$), and yield ($r = 0.00$, $p = 0.99$). Seed length had positive correlation with seed width ($r = 0.96$, $p < 0.001$), seed thickness ($r = 0.96$, $p < 0.001$), and yield ($r = 0.74$, $p < 0.001$), while seed width had a positive correlation with seed thickness ($r = 1.0$, $p < 0.001$) and yield ($r = 0.77$, $p < 0.001$). Finally, seed thickness had positive correlation with yield ($r = 0.76$, $p < 0.001$) in addition to the other correlations earlier reported (**Supplementary Tables S2**, **S3**; **Figure 4**).

Analysis of Genetic Components

The output of genetic components analysis was compiled in **Table 7**. Expectedly, the phenotypic variance was higher than the genotypic variance in all the traits. Yield (kg ha^{-1}) reported higher phenotypic (19,476.39) and genotypic (5,159.09) variances, while the lower phenotypic (0.68) and genotypic (0.23) variances were observed in leaf width. The traits, such as leaf length (LLE; GCV 7.18, PCV 19.95), germination count (GCT; GCV 6.50, PCV 19.61), days to flowering (DTF; GCV 6.31, PCV 10.81), seed length (SEEDL; GCV 14.41, PCV 18.19), seed width (SEEDW; GCV 11.84, PCV 16.13), and seed thickness (SEEDT; GCV 13.48, PCV 17.66), showed below 20% of PCV and GCV. Improvement of this crop can be based on traits with GCV $\geq$ 20% [number of petioles (NPET), number of pods (NPOD), hundred seed weight (HSWT), and Yield) which indicated high variability among these traits although they are influenced by additive genes. Due to the lower GCV values ($\leq$ 10%), LLE,

TABLE 6 | Trait contributions, eigenvalues, and cumulative percentage of the components.

	PC1	PC2	PC3
PH	3.78	9.12	10.80
LLE	0.17	4.16	10.57
LWI	4.92	2.34	11.87
DTE	0.11	3.14	5.74
DT50G	0.01	4.53	3.55
CHLCON	0.05	3.29	0.09
GCT	1.06	3.78	1.33
DTF	0.13	0.56	21.25
NPET	0.01	2.53	3.87
PEL	1.54	8.93	14.92
NPOD	2.33	18.48	2.93
NSEED	0.96	21.78	1.00
HSWT	16.98	0.14	3.98
TSWT	2.75	13.96	4.96
SEEDL	15.93	0.22	0.16
SEEDW	19.93	0.22	0.32
SEEDT	19.58	0.13	0.34
YIELD	9.76	2.68	2.30
Eigenvalue	4.44	3.17	2.19
Percentage variance	24.67	17.63	12.19
Percentage cumulative variance	24.67	42.3	54.49

PH, plant height (cm); LLE, leaf length (cm); LWI, leaf width (cm); DTE, days to emergence; DT50G, days to 50% germination; CHLCON, chlorophyll content; GCT, germination count; DTF, days to flowering; NPET, number of petioles; PEL, petiole length (cm); NPOD, number of pods; NSEED, number of seeds; HSWT, hundred seed weight (g); TSWT, total seed weight (g); SEEDL, seed length (cm); SEEDW, seed width (cm); SEEDT, seed thickness (cm), Yield (kg/ha).

days to emergence (DTE), days to 50% germination (DT50G), GCT, and DTF indicated the limited chance of selection due to environmental effect on their phenotypic expression.

The values of heritability in broad sense were low for most of the traits (**Table 7**), which ranged from 4% (DT50G) to 63% (SEEDL). Very high (≥ 60%) heritability was measured for SEEDL (63%), indicating that this is the only trait that was highly heritable in this study. The heritability value 30–60% was marked for leaf width (LWI, 32%), DTF (34%), SEEDW (54%), and SEEDT (58%) which indicated that the traits were moderately heritable whereas plant height (PH, 29%), HSWT (27%), yield (26%), and the remaining traits showed heritability below 30%, i.e., low heritability. The trait NPET (26.11%) had highest genetic advance (as percentage mean) value (≥ 20%) while the lowest was 1.83% for DT50G (**Table 7**). Remarkably, the higher genetic gain was recorded for grain yield (76.15%), and NPODS (43.08%).

DISCUSSION

High nutrient uptake, the ability to compete favorably with weeds, and yield improvement are some of the prerequisites for developing high yield crops. The significant differences for the different traits scored on the accessions indicate the existence of variability in the selected population that can be exploited for an improved breeding program. Trait variability facilitates trait-assisted selection of best lines for improvement (Moradi et al., 2019; Dewi et al., 2020). Various studies have shown GEI effect on several crops, such as Rice (Rahman and Shah, 2019; Calayugan et al., 2020), sweet potato (Ngailo et al., 2019), and Sorghum (Jiang et al., 2020). Yan and Kang (2003) stated that the number of genotypes and environments determines the extent of environmental variation. They also pointed out that many genotypes in few numbers of environments reduce the environmental variance and *vice versa*. However, according to Aremu et al. (2019), the environment is always the dominant source of variation, and it must be prioritized in plant breeding. Plant germplasm possesses a high degree of variability in its morphology. This high range of variability is harnessed to develop improved cultivars in major crops. Less known or underutilized crops need to benefit from this development too, as they also have a high level of diversity between (inter-variation) and within (intra-variation) their accessions. These are, however, further seen in the effects of various locations where they are cultivated. Therefore, morphological characterization in various locations assists breeders in selecting superior lines for further improvement (Peratoner et al., 2016; Moradi et al., 2019; Dewi et al., 2020).

Bambara groundnut (BGN) is a rich source of diversity as it has been localized in various environments and its relevance, especially in sub-Saharan Africa, is increasing greatly. In this study, all accessions used showed a high level of diversity for all traits studied. This result supports that of other studies that report high variability in BGN accessions (Mohammed et al., 2016; Atoyebi et al., 2017a; Gbaguidi et al., 2018). In the studies carried out by Mohammed et al. (2016), Gbaguidi et al. (2018), and Atoyebi et al. (2017a), the authors recorded CV ≥ 20% for petiole length, number of pods, hundred seed weight, and yield. The high coefficient of variation observed in some of the traits in this study shows a high level of heterogeneity for these traits among the accessions studied (**Table 4**). High heterogeneity in BGN was also reported by Goli et al. (1997), Khan et al. (2020), and Khan et al. (2021). Diversities in morphology result from differences in the genetic make-up (Manimekalai et al., 2018; Ibrahim et al., 2019) of the crop and the likely impact of the locations and planting seasons. We can also attribute these diversities to the inconsistencies observed in traits like DTF, which are affected by day-light length. In this study, the reported DTF were between 28 and 49 days, but Khan et al. (2021) reported 36–53 days, Masindeni (2006) reported 43–80 days, and Goli et al. (1997) reported 38–68 days.

Genotype x environment interactions (GEI) reduces the relationship between genotype and phenotype (Voss-Fels et al., 2019), thereby reducing the progress of selection. Hence, there is a need to study GEI for an effective breeding program for BGN to enable breeders to identify locations that are good representatives of target regions of interest (Gupta et al., 2015). Plant genotypes often tested in different locations and years are affected by differences in soil fertility, climatic factors (such as rainfall, humidity, and temperature), pests, and diseases. This study showed that plant growth-trait responses, especially the

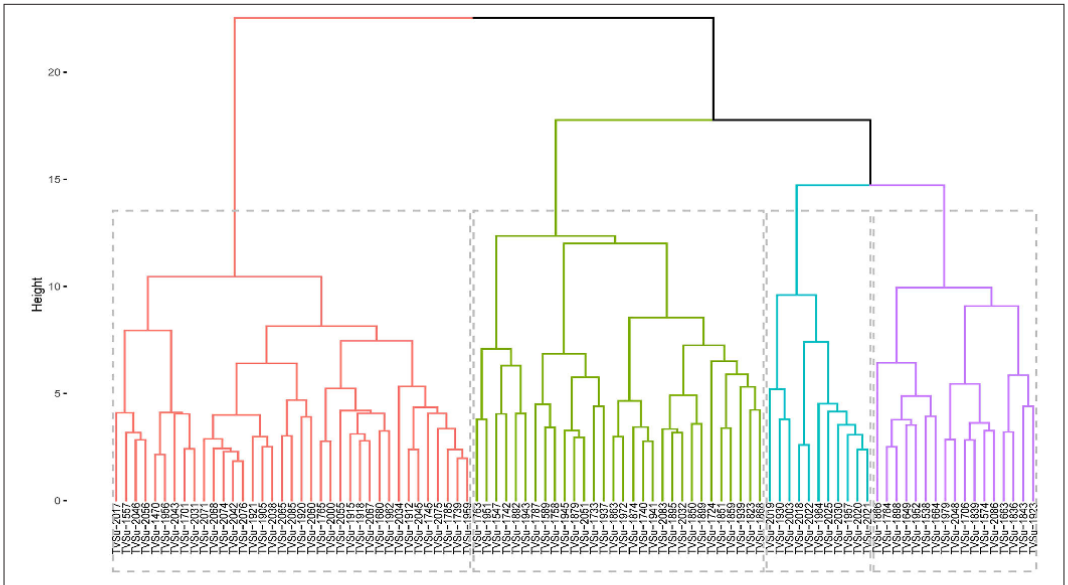

FIGURE 3 | Hierarchical clustering dendrogram analysis; Euclidean distance was used and the associations between groups were done by the Ward method for morphological traits across the environments.

architectural traits, are affected by accessions, locations, and years (**Table 5**). This finding is supported by the result obtained in the study carried out by Mogale (2018) at three different locations in South Africa. The accessions responded differently to each trait at the different locations and years, thus suggesting the existence of GEI (**Supplementary Table S1**). The highly significant location effect observed for all traits (except plant height and leaf length), can be attributed to differences in the climatic and soil conditions exhibited at the two locations (**Tables 2, 3**). This suggests the importance of assessment of accessions under different environments to identify better performing accessions. Effects of GEI on crop evaluation have been reported in various studies (Aarthi et al., 2020; Rubilar et al., 2020; Olanrewaju et al., 2021). However, the accessions showed significant variation in plant height, which is contrary to the reports of Ntundu et al. (2006) in Tanzania and Shegro et al. (2013) in South Africa. The yield and yield-related traits in this study showed a high genetic discrepancy. A similar report was given by Shegro et al. (2013), and these variations were accredited to the effect of genotype by environment interaction on BGN yield. The hundred seed weight is critical for determining morphological traits related to plant yield (Gerrano et al., 2015; Khan et al., 2021). The yield of BGN was recorded from 146.6 to 2678.6 kg ha^{-1} by Gbaguidi et al. (2018) and 1,058.8 kg ha^{-1} by Dansi et al. (2012), whereas in this study, we report from 0 to 1,266.67 kg ha^{-1}. Typically, the Food and Agriculture Organization (FAO; http://www.fao.org/faostat/en/#data/QC) estimated the average yield of BGN (1,180 kg ha^{-1}) is lower than our estimated yield. Variation in seed length and seed width, as observed in this study, may be due to different seed shapes, while variation in hundred seed weight can be attributed to different seed sizes. The findings from this study and other studies show a high level of diversity and a high influence of the environment on the growth and yield of BGN.

Different algorithms, such as multidimensional scaling, clustering, and PCA, are used to assess variability and genetic diversity in various studies (Franco-Duran et al., 2019; Zarei et al., 2019; Munir et al., 2020). In PCA biplot, traits are superimposed on the plot as vectors. Biplot represents the association among different traits and accessions, and the length of the vectors show the contribution of each trait to the observed variations. The eigenvalues and the corresponding factors are sorted by descending order of how much of the initial variability they represent. The Eigenvalue significance criterion, as described by Kaiser (1960), was used to select statistically significant principal components. Among 10 components, the 2 selected showed a value of more than 1. The highest variation observed is represented in the first axis (Iezzoni and Pritts, 1991). In this study, the first component (PC1) accounted for a greater proportion of the variation than the second component (**Table 6**; **Figure 2**). Similar results were reported by Khan et al. (2021) of total variations at 45.88% (PC1) and 10.68% (PC2) in BGN. Several studies also support these findings (Farhad et al., 2008; Usman et al., 2014; Atoyebi et al., 2017a; Mohammed et al., 2020). In addition, PC1 is the most powerful criterion for selection for yield improvement (Adeoti et al., 2012). In this study, leaf width, hundred seed weight, seed width, seed thickness, seed length, and

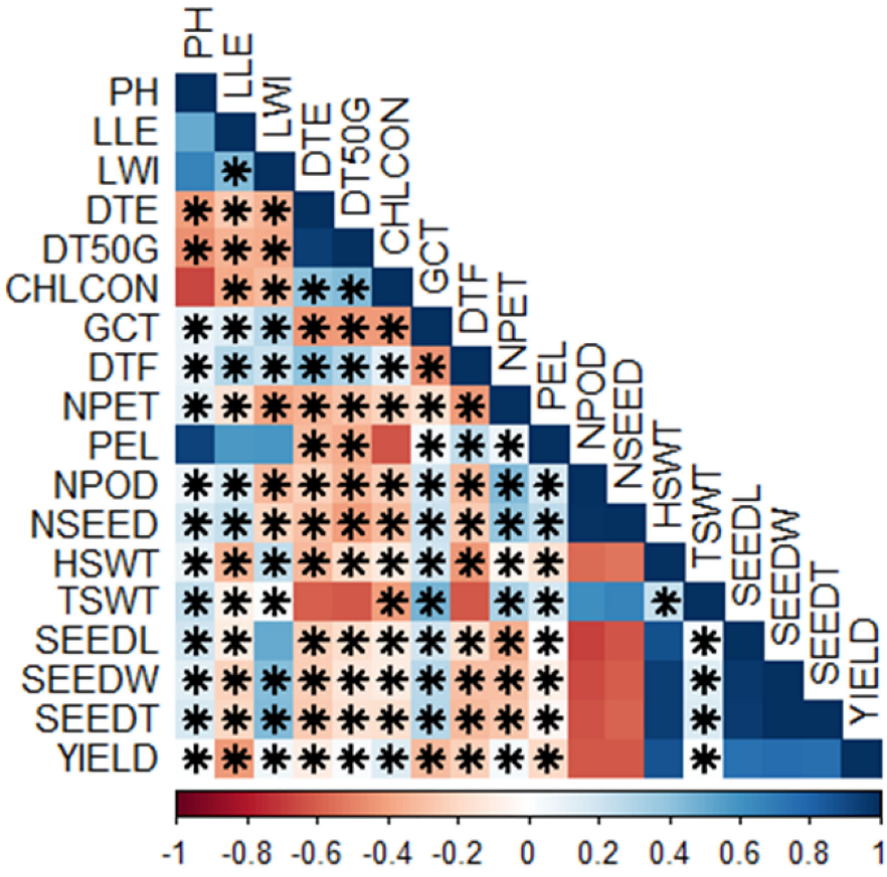

FIGURE 4 | Correlations among the traits scored; Pearson's rank correlation matrix and performance analytic chart of the variables showing the relationship among the variables scored on BGN accessions grown across two locations and years. (Blue color indicates a significant positive correlation and red color indicates a significant negative correlation among different traits. PH, plant height (cm); LLE, leaf length (cm); LWI, leaf width (cm); DTE, days to emergence; DT50G, days to 50% germination; CHLCON, chlorophyll content; GCT, germination count; DTF, days to flowering; NPET, number of petioles; PEL, petiole length (cm); NPOD, number of pods; NSEED, number of seeds; HSWT, hundred seed weight (g); TSWT, total seed weight (g); SEEDL, seed length (cm); SEEDW, seed width (cm); SEEDT, seed thickness (cm), Yield (kg/ha). *Significant, $p < 0.05$.

yield have more influence on PC1. Similar findings were reported by Stoilova and Pereira (2013), Ridzuan et al. (2019), and Khan et al. (2021).

From the biplot (**Figure 2**), accessions that are tall have longer petioles and longer and wider leaves, but these same accessions are low in chlorophyll content and number of petioles. They do not germinate well-compared to the other accessions in the other principal components, they emerge late, and take a longer time to flower and *vice versa*. This study did not determine whether these tall accessions with longer petioles and longer and wider leaves take longer to mature. But we can conclude from the findings that the tall plants should attain maturity earlier than the short plants because the tall plants flower early, which means that they enter the vegetative stage earlier than the short plants. Similar correlations have been reported in Chickpea where the tall plants flower earlier than the short plants (Mallikarjuna et al., 2019). Likewise, accessions that have a higher number of petioles and leaves, flower late, and have poor germination. Chlorophyll content correlates negatively with plant height, but chlorophyll aids in the process of photosynthesis. It might be expected that tall plants have high chlorophyll content. However, short plants with more green leaves will have more chlorophyll

TABLE 7 | Variance components, heritability, and genetic advance for growth and yield traits.

Traits	V_P	V_G	GCV (%)	PCV (%)	h^2 (%)	GA	GAM
PH	48.56	14.21	14.86	27.47	29	4.20	16.56
LLE	1.66	0.21	7.18	19.95	13	0.32	5.33
LWI	0.68	0.23	16.74	29.65	32	0.54	19.48
DTE	3.48	0.31	7.33	24.47	9	0.35	4.52
DT50G	6.00	0.25	4.32	20.97	4	0.21	1.83
CHLCON	159.36	42.84	16.45	31.74	27	6.99	17.57
GCT	2.58	0.61	6.50	19.61	23	0.78	9.47
DTF	16.99	5.78	6.31	10.81	34	2.89	7.57
NPET	2200.51	569.26	24.92	49.00	26	25.00	26.11
PEL	17.45	4.12	12.46	25.64	24	2.03	12.47
NPOD	35373.05	3932.95	32.55	97.61	11	43.08	22.36
NSEED	26053.10	1256.54	19.24	87.61	5	16.04	8.70
HSWT	923.32	248.87	20.90	40.26	27	16.87	22.35
TSWT	9870.43	631.61	16.37	64.72	6	13.10	8.53
SEEDL	4.41	2.77	14.41	18.19	63	2.72	23.53
SEEDW	2.19	1.18	11.84	16.13	54	1.64	17.89
SEEDT	2.81	1.64	13.48	17.66	58	2.01	21.20
YIELD	19476.39	5159.09	22.65	44.01	26	76.15	24.01

V_G, genotypic variance; V_P, phenotypic variance; h^2, heritability in broad sense; PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of variation; GA, genetic advance; GAM, genetic advance of the mean; PH, plant height (cm); LLE, leaf length (cm); LWI, leaf width (cm); DTE, days to emergence; DT50G, days to 50% germination; CHLCON, chlorophyll content; GCT, germination count; DTF, days to flowering; NPET, number of petioles; PEL, petiole length (cm); NPOD, number of pods; NSEED, number of seeds; HSWT, hundred seed weight (g); TSWT, total seed weight (g); SEEDL, seed length (cm); SEEDW, seed width (cm); SEEDT, seed thickness (cm), Yield (kg/ha).

content than tall plants with fewer green leaves. Hence, the number of green leaves, not the height of a plant, should be used to determine the chlorophyll content of a plant. This result is supported by the findings in the studies of Anwar et al. (2020) and Klaassen et al. (2020). They reported that taller plants have a lesser amount of chlorophyll content compared to shorter and younger plants. The positive relationship between the number of pods, number of seeds, and total seed weight highlights the importance of pods and seeds to the total seed weight but not yield, as this study shows. However, hundred seed weight and seed characteristics, on the other hand, have positive impacts on yield. Hence, they are good targets for yield improvement programs. Seed characteristics contribute to the yield more than pods, basically due to postharvest factors such as damaged seeds, which is the most important factor in postharvest yield loss. This accounts for the disparity in the relationship between the number of pods, the number of seeds harvested, and the eventual yield obtained after processing of the seeds.

According to Valombola et al. (2019), similarities in accessions may result from the same accessions bearing different names due to different sources of cultivation. They, however, suggested that breeders should select accessions from different clusters to maximize heterosis. The cluster analysis shows the dendrogram which looks at the combined locations and years (**Figure 3**). The accessions in the first cluster (red segment) are characterized by taller plants, longer leaf length, longer leaf width, high germination rate, longer petioles, high seed weight, longer, wider, and thicker seeds, and are high yielding. The second cluster (green segment) is characterized by accessions having an early emerging date, high in chlorophyll, and flowering early, while the last cluster (blue segment) is majorly characterized by accessions having a high number of pods and number of seeds. This supports the findings of the principal component analysis.

In the correlation matrix (**Figure 4**), the positive correlation between the numbers of pods, number of seeds, and total seed weight indicates the importance of the number of pods and seeds on the total seed weight. However, this does not affect the yield as they are negatively correlated to the yield. The traits correlating positively with yield in this study are the same as those reported in the study of Gbaguidi et al. (2018) except for the number of seeds, which is negatively correlated in this study but positively correlated in their study. From this study, important traits for yield improvement are seed parameters and a hundred seed weight. Environmental variations also indicate the role of the environment on yield and related traits. A better understanding of the relationship between the yield-related components will provide an appropriate way of improving the yield of crops. Correlation among different components indicates the complementary functional roles of these traits on grain yield and their adaptability to different locations. The positive correlation between seed length and seed width will be crucial for farmers as they will prefer seeds with big size. Therefore, these two parameters are important for size improvement. The result from the study of Valombola et al. (2019) supports this result.

The result of the genetic parameters showed higher values for phenotypic than genotypic variances for all traits, implying the influence of the environment on the expressed traits. This result is in line with the reports of Onwubiko et al. (2019) and

Khan et al. (2021). GCV and PCV values were categorized based on the suggested index of 0-10% for low, 10–20% for moderate, and ≥ 20% for high variation (Khan et al., 2020). Based on this category, genetic component analysis showed that GCV was high for number of petioles, number of pods, and hundred seed weight and yield, while PCV was high for most of the traits. Traits with reasonable variations present a wide opportunity for improvement (Onwubiko et al., 2019) while traits exhibiting low GCV and PCV show low variability, hence, they cannot be used to discriminate among the accessions. Therefore, they cannot be used for selection for crop improvement. Considering heritability and genetic advance, none of the traits in this study show both high heritability and genetic advance, which implies that direct selection cannot be recommended because of the low addictive gene effect. Low to moderate heritability and genetic advance values can hinder the use of traits for selection purposes due to high environmental effects over genetic effects (Ridzuan et al., 2019). Therefore, effective selection can be accomplished by picking traits with high GCV, PCV, heritability, and genetic advance (Usman et al., 2014). Selection of traits with low heritability and genetic progress should be delayed until their genetic effects outweigh their environmental effects (Onwubiko et al., 2019).

Furthermore, molecular characterization of the selected accessions will help to identify similarities and therefore, prevent selection of the same accessions for improvement. This will help in eliminating duplication of accessions during breeding programs. This study focuses on two locations and a few numbers of accessions. Therefore, highly contrasting environments can be used in the trial to give a more rounded and concrete locational response. Multilocational trials should be carried out on large germplasm collections to extensively detect most of the variations in BGN, followed by a selection of the best accessions as regards a trait of interest based on the objectives of the researchers to develop new and improved varieties of the crop.

CONCLUSION

Expressions of genes that regulate various traits are subject to modification by the environment because different accessions show variations in their responses to different environments. In improvement programs, accessions are tested for their performance for some years and at multiple locations to select high-quality accessions. Though BGN has been reported to thrive in various weather conditions, its performance can be largely hampered by various environmental factors such as soil fertility, rainfall, temperature, and day length period. These factors constitute the effect of GEI on plant growth traits. More research on this crop will go a long way in achieving food security, especially in sub-Saharan Africa. Some accessions in this study, mostly those originating from southern Africa, show great promise for the development of varieties with improved phenotypic, vegetative, and yield traits. The performance of these accessions in both locations and their combined performances are good criteria for selection purposes. Low to moderate values for heritability and genetic advance in most traits studied indicated that selection from these accessions based on genetic effect is not yet possible.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

OSO, OO, and MA designed the experiment. OSO carried out the trials, collected the data, performed the analysis, and wrote the manuscript. OOB, OO, and MA supervised the study and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.796352/full#supplementary-material

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CHAPTER 4: ARTICLE 3

GGE Biplot Analysis of Genotype x Environment Interaction and Yield Stability in Bambara Groundnut

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GGE Biplot Analysis of Genotype × Environment Interaction and Yield Stability in Bambara Groundnut

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Abstract: In plant breeding and agricultural research, biplot analysis has become an important statistical technique. The goal of this study was to find the winning genotype(s) for the test settings in a part of the Southwest region of Nigeria, as well as to investigate the nature and extent of genotype × environment interaction (GEI) effects on Bambara groundnut (BGN) production. The experiment was carried out in four environments (two separate sites, Ibadan and Ikenne, for two consecutive years, 2018 and 2019) with ninety-five BGN accessions. According to the combined analysis of variance over environments, genotypes and GEI both had a substantial ($p < 0.001$) impact on BGN yield. The results revealed that BGN accessions performed differently in different test conditions, indicating that the interaction was crossover in nature. The results revealed that BGN accessions performed differently in different test conditions, indicating that the interaction was crossover in nature. To examine and show the pattern of the interaction components, biplots with the genotype main effect and genotype × environment interaction (GEI) were used. The first two PCs explained 80% of the total variation of the GGE model (i.e., G + GE) (PC1 = 48.59%, PC2 = 31.41%). The accessions that performed best in each environment based on the "which-won-where" polygon were TVSu-2031, TVSu-1724, TVSu-1742, TVSu-2022, TVSu-1943, TVSu-1892, TVSu-1557, TVSu-2060, and TVSu-2017. Among these accessions, TVSu-2017, TVSu-1557, TVSu-2060, TVSu-1892, and TVSu-1943 were among the highest-yielding accessions on the field. The adaptable accessions were TVSu-1763, TVSu-1899, TVSu-2019, TVSu-1898, TVSu-1957, TVSu-2021, and TVSu-1850, and the stable accessions were TVSu-1589, TVSu-1905, and TVSu-2048. In terms of discriminating and representativeness for the environments, Ibadan 2019 is deemed to be a superior environment. The selected accessions are recommended as parental lines in breeding programs for grain yield improvement in Ibadan or Ikenne or similar agro-ecological zones.

Keywords: Bambara groundnut; food security; genotype × environment interaction; GGE biplot; multi-environment trial; stability analysis; yield

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1. Introduction

Bambara groundnut (BGN) belongs to the family *Fabaceae* and is commonly grown in sub-Saharan Africa and some parts of Asia [1]. The pod, seed qualities, plant vigor, plant spread, leaf shape, nutritional, and antinutrient components all have a wide range of diversity. Farmers, particularly those in rural communities, benefit from it as a source of revenue. It can be utilized as a human food source and as a livestock feed additive. Reports have emerged that it can be used in the treatment of diseases such as diarrhea [2]. The seeds are rich in protein, carbohydrates, fat, mineral content, and fiber [3,4]. BGN is believed to be the most resilient to drought among grain legumes [5,6]. Wild varieties predominate due to limited research into domesticating new varieties. The major producing/exporting

countries are Niger, Ghana, Chad, Nigeria, Mali, Senegal, Côte d'Ivoire, Burkina Faso, and Togo. Some parts of southern Africa also grow the crop; however, Purseglove [7] reported that the most extensive production of the crop in southern Africa is in Zambia.

BGN seeds are not sold on world markets as they are not widely accepted as a major crop yet, but these seeds are an important part of the diet in several countries in West Africa, where they are highly valued. Their consumption in this region is 3rd to only cowpea and peanut according to the national production and consumption statistics [8]. In Ghana specifically, the seeds are canned, which makes them available throughout the year. Over 40,000 cans of different sizes are available. The seeds can be consumed when they are still immature or when fully matured, although matured seeds are hard and not easy to cook; they can, however, be ground into powder before further processing for consumption [9]. To soften them more quickly, they can also be boiled until soft after soaking for a while.

The International Institute of Tropical Agriculture, in Nigeria, retains over 1900 accessions obtained from various countries in their Genetic Resources Center [10]. BGN seeds are nutrient-dense and economically important. Demand for the seeds is high, and supply is limited in their area of cultivation [11]. Despite these benefits, BGN's agro-ecological and genetic potential remains untapped. Instead of agro-ecological or production-specific varieties, it is still grown from local landraces.

Despite intensive national breeding efforts for BGN, there are few studies on yield stability in this agricultural zone, which limits the sustainability of sufficient grain production. Even though BGN yield varies greatly among landraces, no landrace can be considered a true variety [12]. The situation can be improved by breeding crops that produce high yields in a wide range of agro-climatic conditions. However, the potential large interaction with a complex set of bio-physical environments prevailing in a region complicates direct selection for yield in the field [13]. To effectively identify the yield potential of consistently performing BGN accession, yield and stability must be considered simultaneously in a variety of accessions.

Its yield stability and adaptability determine any crop variety's ability to thrive in a given environment. Due to differences in the various environments, these traits are influenced by genotype × environment interactions (GEI). Plant breeders are increasingly interested in GEI to identify long-term solutions to issues controlling plant growth and development. Because of the increasing interest, several statistical methods have been developed for multi-environment trials (MET) to study GEI effects [14,15]. The two most common methods used for MET are additive main effects and multiplicative interaction (AMMI), and genotype plus genotype environment interaction (GGE) biplot [16]. Both methods are used for straightforward graphical representation of a complex genotype by two-way environment tables using principal component analysis [17]. The difference between the two methods is based on how the means are treated before the singular value decomposition (SVD) is performed. With AMMI, SVD is applied to the data excluding genotype and environment means, while GGE biplot excludes from the data the environment means only [18]. According to Alizadeh et al. [16], both methods are highly correlated, and therefore can be used interchangeably.

The biplot and the GGE concepts are used in the GGE biplot method to visually analyze the results of site regression analysis in MET data [19]. The concept of GGE biplot involves the use of biplot to show the two important factors, which are also sources of variation (viz., G and GE). GGE biplot fits best for genotype evaluation (mean vs. stability), test environments which provide discriminating power vs. representativeness, and multi-environment analysis (such as "which-won-where" pattern) [20,21]. GGE biplot is a versatile method with the ability to analyze a range of data types using a two-way structure [22]. Since the introduction of the GGE biplot, numerous applications of the method on MET analysis have been reported. Alake et al. [23] determined the yield stability of 24 BGN landraces using GGE biplot analysis. Elsewhere, GGE biplot analysis of yield stability for Andean dry bean accessions grown under different abiotic stress regimes in Tanzania was reported by Mndolwa et al. [24]. Soybean performance and stability in

MET using GGE biplot analysis was also reported by Dalló et al. [25]. Other applications of GGE biplot have been reported on various crops such as maize [26], sugarcane [27], sunflower [28], rice [17], and wheat [29].

The goals of this study were to examine and quantify the magnitude of GEI effects on BGN yield, as well as to determine the adaptability and stability of the 95 BGN accessions in the studied test environments in Southwest Nigeria.

2. Materials and Methods

2.1. Study Site Descriptions

The study was conducted in two different agroecological zones: Ibadan (7°40′19.62″ N, 3°91′73.13″ E), which is a derived savannah, and Ikenne (6°51′00.873″ N, 3°41′48.528″ E), which is a rain forest. International Institute of Tropical Agriculture (IITA) field stations in Ibadan and Ikenne were used. The study was carried out in the 2018 and 2019 planting seasons. The average climate data for the study sites are shown in Table 1.

Table 1. Monthly mean meteorological data of the experimental sites during BGN growing season (average of 2018–2019 and 2019–2020 crop season).

			August	September	October	November	December	January
Ibadan	2018/2019	Average temperature (°C)	25	25	25	30	30	29
		Average precipitation (mm)	94.8	99.2	53.5	3.3	0	37.6
		Average relative humidity (%)	85	87	86	69	53	62
	2019/2020	Average temperature (°C)	26	26	26	28	29	29
		Average precipitation (mm)	266.7	319.8	661	69.8	1.3	0.9
		Average relative humidity (%)	82	83	85	77	62	46
Ikenne	2018/2019	Average temperature (°C)	26	26	27	28	29	29
		Average precipitation (mm)	113.2	163.9	69.3	13.3	0.6	45.1
		Average relative humidity (%)	87	90	87	86	79	82
	2019/2020	Average temperature (°C)	26	26	26	28	28	28
		Average precipitation (mm)	390.3	300.9	565.8	145.4	12.7	1
		Average relative humidity (%)	85	88	89	85	83	71

2.2. Soil Sampling and Analysis

Topsoil samples were obtained from 0–15 cm over the entire plot using a soil auger and put together to obtain a composite sample before establishing the experiment after the harvest. The soil sample was dried under shade, and passed through a 2 mm sieve for subsequent chemical analyses (sand, clay, silt, pH, organic carbon (OC), total N, exchangeable Ca, Mg, K, available P, Na, Mn, Cu, Fe, and Zn) and particle size distribution at the onset of the experiment.

2.3. Plant Materials, Field Trials, and Yield Data Collection

A set of 95 accessions out of the BGN germplasm that was housed at the Genetic Resources Centre, IITA, Ibadan, Nigeria, was used in this study (Table 2).

The accessions were evaluated using randomized complete block design. Sixty seeds of each accession were planted during the 2018 and 2019 planting seasons. The accessions were planted in three replicates, with each replicate having 20 plants per accession on a plot, which were later thinned to 10 plants at 2 weeks after emergence. The length of each plot was 3 m, with 0.3 m spacing between each plant and a row spacing of 0.7 m between each plot. Each replicate contained 3 blocks, which were separated by 1 m spacing, and the replicates were separated from one another by 2 m spacing. The first planting in 2018 was on 1 and 12 September, while that of the 2019 season was on 26 August and 16 September, in Ikenne and Ibadan, respectively. Plants were rain fed until the stop of rain, then irrigation was applied once a week until harvest. The 2018 planting was harvested on 2 and 18 January 2019, while the 2019 harvesting was done on 11 and 15 January 2020, for

Ikenne and Ibadan, respectively. Seeds were weighed to obtain the total seed weight per plot, which was then converted to kg/ha using the following formula:

$$\text{Yield(kg/ha)} = \frac{\text{plot yield} * 10,000}{\text{plot area}}$$

Table 2. Bambara groundnut accessions and their origin.

	Accessions	Passport Data		Accessions	Passport Data		Accessions	Passport Data
1	TVSu-1470	Ghana	33	TVSu-1866	Zimbabwe	65	TVSu-2017	Burundi
2	TVSu-1538	unknown	34	TVSu-1868	Zimbabwe	66	TVSu-2018	Burundi
3	TVSu-1547	unknown	35	TVSu-1874	Botswana	67	TVSu-2019	Burundi
4	TVSu-1557	unknown	36	TVSu-1879	Botswana	68	TVSu-2020	unknown
5	TVSu-1574	unknown	37	TVSu-1892	Botswana	69	TVSu-2021	unknown
6	TVSu-1589	unknown	38	TVSu-1895	Botswana	70	TVSu-2022	unknown
7	TVSu-1649	Senegal	39	TVSu-1898	Unknown	71	TVSu-2025	unknown
8	TVSu-1663	Senegal	40	TVSu-1899	Unknown	72	TVSu-2030	unknown
9	TVSu-1664	Senegal	41	TVSu-1905	Unknown	73	TVSu-2031	unknown
10	TVSu-1680	Togo	42	TVSu-1912	Cameroon	74	TVSu-2032	unknown
11	TVSu-1701	Togo	43	TVSu-1915	Cameroon	75	TVSu-2034	unknown
12	TVSu-1706	Zambia	44	TVSu-1918	Cameroon	76	TVSu-2038	unknown
13	TVSu-1724	Zambia	45	TVSu-1920	Senegal	77	TVSu-2042	unknown
14	TVSu-1733	Zambia	46	TVSu-1921	Malawi	78	TVSu-2043	unknown
15	TVSu-1739	Zambia	47	TVSu-1923	Zimbabwe	79	TVSu-2045	unknown
16	TVSu-1740	Zambia	48	TVSu-1930	Zimbabwe	80	TVSu-2046	unknown
17	TVSu-1742	Zambia	49	TVSu-1937	Zimbabwe	81	TVSu-2048	unknown
18	TVSu-1745	Malawi	50	TVSu-1939	Zimbabwe	82	TVSu-2051	unknown
19	TVSu-1758	Malawi	51	TVSu-1941	Zimbabwe	83	TVSu-2055	unknown
20	TVSu-1763	Malawi	52	TVSu-1943	Zimbabwe	84	TVSu-2056	unknown
21	TVSu-1764	Malawi	53	TVSu-1945	Zimbabwe	85	TVSu-2060	unknown
22	TVSu-1765	Malawi	54	TVSu-1951	Zimbabwe	86	TVSu-2065	unknown
23	TVSu-1785	Malawi	55	TVSu-1952	Zimbabwe	87	TVSu-2067	unknown
24	TVSu-1787	Cameroon	56	TVSu-1956	Zimbabwe	88	TVSu-2068	unknown
25	TVSu-1823	Niger	57	TVSu-1957	Zimbabwe	89	TVSu-2071	unknown
26	TVSu-1836	Niger	58	TVSu-1959	Swaziland	90	TVSu-2074	unknown
27	TVSu-1839	Zimbabwe	59	TVSu-1962	Swaziland	91	TVSu-2075	unknown
28	TVSu-1843	Zimbabwe	60	TVSu-1964	DRC	92	TVSu-2076	unknown
29	TVSu-1850	Zimbabwe	61	TVSu-1972	DRC	93	TVSu-2083	unknown
30	TVSu-1851	Zimbabwe	62	TVSu-1979	Burundi	94	TVSu-2085	unknown
31	TVSu-1859	Zimbabwe	63	TVSu-2000	Burundi	95	TVSu-2086	unknown
32	TVSu-1863	Zimbabwe	64	TVSu-2003	Burundi			

2.4. Statistical Analysis

The analysis of yield data was carried out using the R statistical package [30]. The yield data were subjected to analysis of variance (ANOVA) after the data had been normalized by log-transforming. Each year at each location was considered as a separate environment.

In this study, Eberhart and Russell's joint regression model was used for the stability analysis. Eberhart and Russell's [14] model uses joint linear regression where the yield of each genotype is regressed on the environmental index. The behavior of the genotype was determined by the following model: $Y_{ij} = \mu_i + \beta_i I_j + \delta_{ij}$.

In the above model, Y_{ij} = the mean performance of the ith genotype in the jth environment, μ_i = the grand mean of the ith genotype over all the environments, β_i = the regression coefficient which measures the response of the ith genotype on the environmental index, I_j = the environmental index obtained by the difference between the mean of each environment and the grand mean, and δ_{ij} = the deviation from the regression of the ith variety in the jth environment.

If there was a significant difference in genotype–environment interaction, the GGE biplot method was employed to analyze and assess the interaction and yield stability. The GGE biplot was constructed using the first two principal components (PC1 and PC2) derived using environment-centered yield data [31]. GEA-R version 4.1 [32] was used to analyze GGE biplot and stability analysis. SVD of the first two principal components was used to fit the GGE biplot model [33]:

$$Y_{ij} = \mu + \beta_j + \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + e_{ij}$$

where Y_{ij} is the trait mean for genotype i in environment j; μ is the grand mean; β_j is the main effect of environment j; $\mu + \beta_j$ is the mean yield across all genotypes in environment j; λ_1 and λ_2 are the singular values (SV) for the first and second principal components (PC1 and PC2), respectively; ξ_{i1} and ξ_{i2} are eigenvectors of genotype i for PC1 and PC2, respectively; $\eta_1 j$ and $\eta_2 j$ are eigenvectors of environment j for PC1 and PC2; and ε_{ij} is the residual associated with genotype i in environment j. In GGE biplot analysis, scores of PC1 were plotted against PC2 [20].

Accordingly, GGE biplot analysis was also used to generate graphs for the (i) mean performance and stability analysis, (ii) which-won-where pattern, (iii) relationship among test locations, and (iv) ranking discrimination and representativeness of test locations. Angles between location vectors in GGE biplot were used to judge the correlation between pairs of locations [19].

3. Results and Discussion

3.1. Soil Analysis

Soil physicochemical properties for the two locations in both seasons are presented in Table 3. Higher amounts of sand, calcium, magnesium, potassium, sodium, manganese, iron, copper, and zinc were recorded in Ikenne compared to Ibadan for the 2018 planting season, while in the 2019 planting season, Ibadan had the highest pH, sand, nitrogen, organic carbon, manganese, iron, and zinc when compared with Ikenne (Table 3). Different soil properties were recorded at both locations for both years.

Table 3. Soil properties at the beginning of the experiment for individual locations and seasons.

Properties	2018		2019	
	Ibadan	Ikenne	Ibadan	Ikenne
Sand%	73.67	80.33	79.33	75.00
Clay%	19.67	13.67	14.00	15.67
Silt%	6.67	6.00	6.67	9.33
pH	6.70	6.42	6.59	5.02
%N	0.17	0.10	0.10	0.09
Bray P	13.45	22.48	11.27	18.89
%OC	1.02	0.41	0.44	0.41
Ca (cmol/kg)	1.13	3.53	1.19	3.53
Mg (cmol/kg)	0.07	0.80	0.27	0.80
K (cmol/kg)	0.14	0.56	0.22	0.56
Na (cmol/kg)	0.06	0.08	0.05	0.08
Mn (ppm)	150.39	154.82	135.30	112.15
Fe (ppm)	86.22	85.84	89.46	85.58
Cu (ppm)	0.55	1.17	0.20	1.17
Zn (ppm)	1.05	1.96	2.72	1.96

Crop yield has been shown to be influenced by soil and climatic conditions. Crops respond differently to various soil types [34]. BGN produces well in sandy soils. Even though sandy soil inhibits crop emergence, BGN benefits from it because it bears fruit underground. Sandy soil has a porous structure with large pores, allowing pods to grow. When sandy soils dry out, they produce thin, loose fissures [35]. This is an advantageous

trait, especially in the semi-arid tropics, where rainfall is unpredictable and soil is subjected to prolonged periods of dryness. Although clay soil has a high water-retention capacity, it expands when wet and contracts when dry over long periods of time [36].

3.2. Pooled Analysis of Variance

To check for a significant GEI, analysis of variance was performed (Table 4). Finding the most suitable genotypes for yield improvements is quite difficult due to the GEI's large impact on yield. To produce successful breeding strategies for complex and highly quantitative traits like grain yield in BGN, breeders must quantify GEI. An identifiable and distinct selection pressure was brought to bear in each environment due to varying environmental factors, such as topography and climate. If an adaptation based on environment is ignored, then an overall mean that disregards it would be misleading. Therefore, in order to produce quality results, the method of selection should consider both genotype and environmental factors. According to Bhartiya et al. [37], the number of years of an experiment should be prioritized over the number of locations, and genotype–environment interactions should be considered for the selection of superior genotypes [38,39]. The accessions and environments on their own displayed significant level of variability in their yield responses at $p < 0.001$ and $p \leq 0.05$, respectively, while the GEI effect was significant at $p < 0.001$. This indicates that the accessions do not show consistent performance across the studied environments. The present findings agree with those of Chibarabada et al. [40], who found significant interaction between site and species for BGN grain yield.

Table 4. Analysis of variance for yield data obtained from BGN trials conducted in Ibadan and Ikenne in 2018 and 2019 (environments constitute year–location combinations).

Source of Variations	Df	Sum Sq.	Mean Sq.	F Value	Pr (>F)	
ENV	3	6.146	2.04878	2.9675	0.09717	.
REP(ENV)	8	5.523	0.69041	4.7362	1.10×10^{-5}	***
GEN	94	43.182	0.45938	3.1513	$<2.2 \times 10^{-16}$	***
ENV: GEN	282	55.903	0.19824	1.3599	0.0007	***
Residuals	752	109.622	0.14577			

Coefficient of variation = 0.39. DF = Degree of freedom. Sum sq. = sum of squares. Mean sq. = mean square. . Significant at $p \leq 0.05$. *** Significant at $p < 0.001$.

3.3. Stability Analysis

Following the Eberhart and Russell [14] method, the stability of the accessions across the environments was analyzed (Table 5 and Figure 1). Becker and Léon [41] reported that genotypes having bi = 0 are not affected by environmental factors; thus, they are said to be stable, while those showing average responses possess bi = 1. On the other hand, Eberhart and Russell [14] proposed that genotypes are stable if they show high mean performances, their regression coefficient equals 1, and their deviation from regression is as low as possible.

Coefficient of variation (CV) analysis showed 22 out of the 95 accessions as highly productive and stable (Figure 1A). TVSU-1763, TVSU-1850, TVSU-1898, TVSU-1899, TVSU-1957, TVSU-2019, and TVSu-2021 were the best-adapting accessions, while TVSU-1589, TVSU-1905, and TVSU-2048 were the most stable accessions, according to Eberhart and Russell's model (Figure 1B). In this study, none of the accessions were recorded to be adaptable and stable in the environments tested.

3.4. GGE Biplot Analysis

GGE biplot allows environment evaluation based on the discriminating ability and representativeness of the GGE view [42]. This ability gives it an edge over the AMMI biplot analysis [43]. The GGE biplot analysis was used to identify the best accessions for each environment and assess their stabilities. The relationship among the test environments was modelled based on environment-centered (centering, 2) and environment-metric-

preserving (SVP, 2) without scaling option in the GEA-R software. The biplot explained 80% of the total variation observed, of which 48.59% was explained by the first principal component (axis1), while the second principal component (axis2) explained 31.41%. Any test environment's capability can be visualized by examining the environment's discriminating power and representativeness [44]. The length of the environment vectors is proportional to the standard deviation within each biplot's environment and indicates the environment's ability to discriminate [45]. From the biplot analysis (Figure 2A) and representation of the accessions in the various environments (Figure 2B), none of the environments are close to the mean environment (Figure 2B). Accession performance in each environment is shown in Figure 3. For the four environments, most of the accessions are clustered close to the average yield, especially in both IB2018 and IB2019 environments. IK2018 and IK2019 are also clustered to the average yield but have a clearer distinction than the other two environments.

Table 5. Mean and stability analysis for the accessions based on Eberhart and Russell method.

Accessions	Mean	Sd	CV (%)	bi	S2di	Accessions	Mean	Sd	CV (%)	bi	S2di
TVSu-1470	8.32	0.36	4.35	0.42	0.15	TVSu-1937	8.26	0.27	3.27	1.75	0.03
TVSu-1538	8.12	0.23	2.82	1.44	0.01	TVSu-1939	8.16	0.08	0.94	−0.03	−0.04
TVSu-1547	8.40	0.26	3.10	1.76	0.02	TVSu-1941	8.05	0.20	2.53	2.08	−0.03
TVSu-1557	8.61	0.34	3.93	−1.91	0.08	TVSu-1943	8.51	0.54	6.39	3.64	0.25
TVSu-1574	8.21	0.28	3.45	2.06	0.03	TVSu-1945	8.13	0.14	1.74	−1.39	−0.04
TVSu-1589	8.29	0.21	2.54	2.46	−0.05	TVSu-1951	8.43	0.24	2.81	2.11	−0.01
TVSu-1649	8.24	0.33	4.00	1.30	0.10	TVSu-1952	8.17	0.50	6.12	3.99	0.15
TVSu-1663	8.33	0.14	1.65	1.35	−0.04	TVSu-1956	8.26	0.22	2.63	−1.70	−0.01
TVSu-1664	8.03	0.34	4.22	0.79	0.12	TVSu-1957	8.15	0.28	3.43	2.60	0.00
TVSu-1680	8.19	0.30	3.65	1.74	0.05	TVSu-1959	8.38	0.19	2.31	1.92	−0.03
TVSu-1701	8.18	0.17	2.04	−0.74	−0.01	TVSu-1962	8.16	0.24	2.98	1.12	0.03
TVSu-1706	8.16	0.11	1.40	0.54	−0.03	TVSu-1964	8.14	0.49	5.96	1.90	0.27
TVSu-1724	7.95	0.25	3.10	2.17	−0.01	TVSu-1972	7.97	0.21	2.67	0.87	0.01
TVSu-1733	8.35	0.18	2.16	−0.56	0.00	TVSu-1979	8.24	0.12	1.50	1.23	−0.04
TVSu-1739	8.36	0.10	1.17	−0.19	−0.03	TVSu-2000	8.20	0.27	3.24	0.92	0.05
TVSu-1740	8.16	0.21	2.52	1.99	−0.03	TVSu-2003	8.50	0.28	3.27	0.30	0.07
TVSu-1742	8.37	0.47	5.58	3.35	0.16	TVSu-2017	8.56	0.39	4.59	−1.40	0.16
TVSu-1745	8.45	0.29	3.46	0.64	0.08	TVSu-2018	8.35	0.27	3.25	2.66	−0.01
TVSu-1758	8.26	0.27	3.24	1.40	0.04	TVSu-2019	8.62	0.30	3.45	2.98	−0.01
TVSu-1763	8.44	0.39	4.63	4.13	0.00	TVSu-2020	8.16	0.33	3.99	1.68	0.08
TVSu-1764	8.09	0.25	3.12	1.51	0.02	TVSu-2021	8.08	0.28	3.44	2.35	0.01
TVSu-1765	8.29	0.18	2.17	1.76	−0.03	TVSu-2022	8.33	0.49	5.93	4.28	0.12
TVSu-1785	8.23	0.12	1.50	0.85	−0.03	TVSu-2025	7.86	0.42	5.33	3.21	0.10
TVSu-1787	8.03	0.35	4.36	1.39	0.11	TVSu-2030	8.13	0.47	5.73	3.49	0.15
TVSu-1823	8.04	0.19	2.32	−0.06	0.00	TVSu-2031	8.26	0.18	2.22	−0.23	0.00
TVSu-1836	8.15	0.14	1.73	1.40	−0.04	TVSu-2032	8.04	0.16	1.96	0.72	−0.02
TVSu-1839	8.28	0.12	1.46	−0.89	−0.04	TVSu-2034	8.18	0.24	2.97	−0.99	0.03
TVSu-1843	8.44	0.29	3.39	−1.98	0.03	TVSu-2038	8.49	0.19	2.20	−1.32	−0.02
TVSu-1850	8.01	0.25	3.16	2.18	0.00	TVSu-2042	8.31	0.24	2.92	−1.52	0.02
TVSu-1851	7.63	0.29	3.80	1.46	0.05	TVSu-2043	8.40	0.25	2.93	−1.06	0.03
TVSu-1859	8.04	0.27	3.32	2.90	−0.03	TVSu-2045	8.39	0.12	1.37	0.57	−0.03
TVSu-1863	8.03	0.33	4.09	2.76	0.03	TVSu-2046	8.60	0.46	5.34	2.43	0.20
TVSu-1866	8.15	0.51	6.24	4.94	0.08	TVSu-2048	8.14	0.05	0.60	−0.16	−0.05
TVSu-1868	7.88	0.20	2.54	0.59	0.01	TVSu-2051	8.19	0.13	1.54	0.91	−0.03
TVSu-1874	8.36	0.08	0.97	0.33	−0.04	TVSu-2055	8.37	0.21	2.47	1.33	0.00
TVSu-1879	7.99	0.20	2.45	0.30	0.01	TVSu-2056	8.62	0.36	4.20	1.15	0.13
TVSu-1892	8.73	0.36	4.10	0.98	0.13	TVSu-2060	8.58	0.30	3.50	−0.79	0.08
TVSu-1895	7.93	0.07	0.83	0.25	−0.04	TVSu-2065	8.27	0.24	2.95	−0.66	0.04
TVSu-1898	8.17	0.29	3.53	2.68	0.00	TVSu-2067	8.27	0.13	1.61	0.64	−0.03
TVSu-1899	8.02	0.35	4.31	3.79	−0.02	TVSu-2068	8.43	0.09	1.12	0.34	−0.04
TVSu-1905	8.39	0.04	0.47	−0.27	−0.05	TVSu-2071	8.46	0.18	2.11	0.67	−0.01
TVSu-1912	8.36	0.05	0.65	−0.13	−0.04	TVSu-2074	8.48	0.21	2.52	−0.98	0.01
TVSu-1915	8.34	0.17	2.04	0.66	−0.01	TVSu-2075	8.42	0.27	3.15	0.18	0.06
TVSu-1918	8.24	0.20	2.43	−1.25	−0.01	TVSu-2076	8.41	0.20	2.39	0.22	0.01
TVSu-1920	8.54	0.12	1.39	−0.31	−0.03	TVSu-2083	8.26	0.12	1.50	0.21	−0.03
TVSu-1921	8.50	0.25	2.91	0.43	0.04	TVSu-2085	8.34	0.26	3.08	0.44	0.05
TVSu-1923	8.53	0.30	3.54	2.15	0.04	TVSu-2086	8.19	0.17	2.07	−0.62	−0.01
TVSu-1930	8.34	0.14	1.68	0.36	−0.02						

Sd = Standard deviation, CV = coefficient of variation, bi = Regression coefficient, S2di = Standard deviation from linearity of regression.

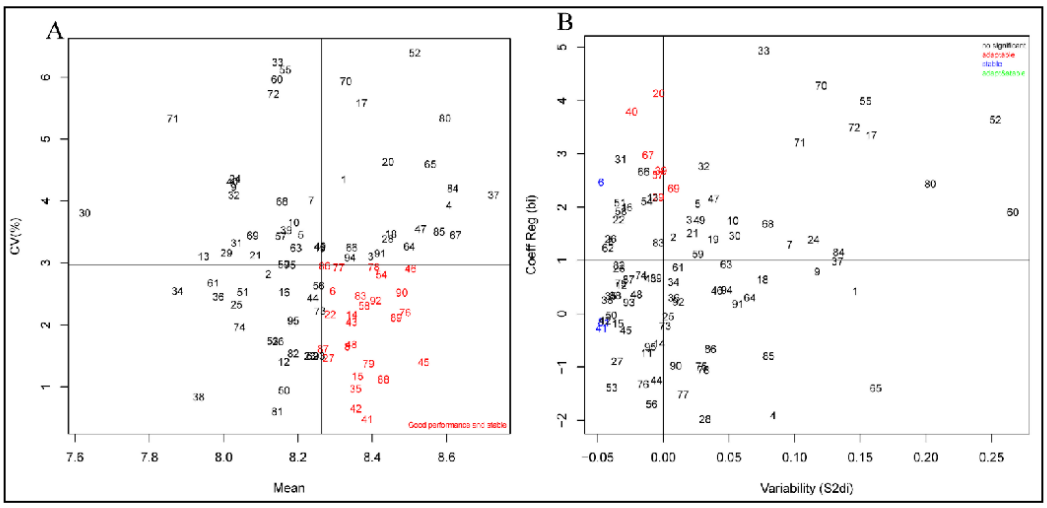

Figure 1. Stability analysis of the accessions with the environments. (**A**) Francis (CV) vs. (**B**) mean biplot for grain yield. Eberhart and Russell (bi, S2di) biplot for grain yield.

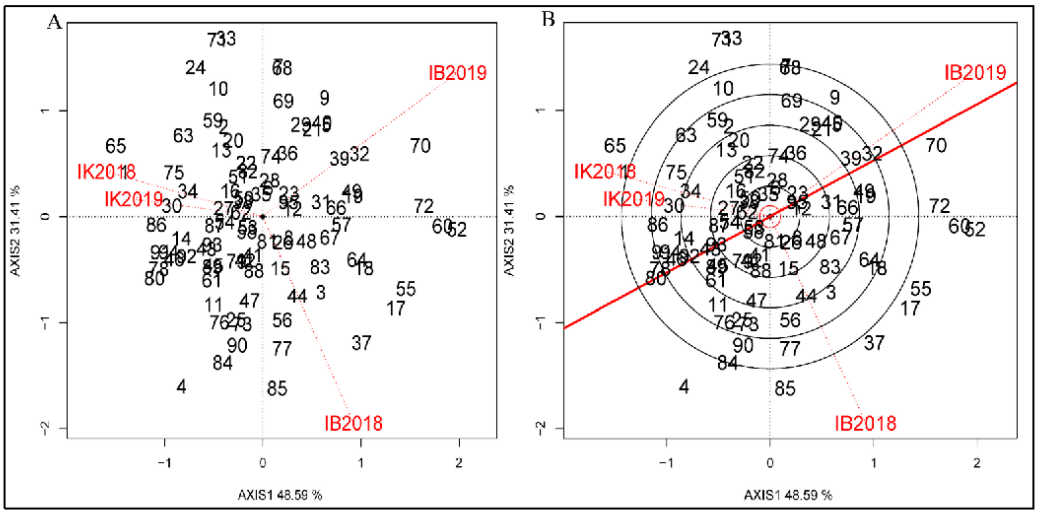

Figure 2. (**A**) Biplot analysis result. (**B**) Discriminativeness and representativeness of the accessions with the environments.

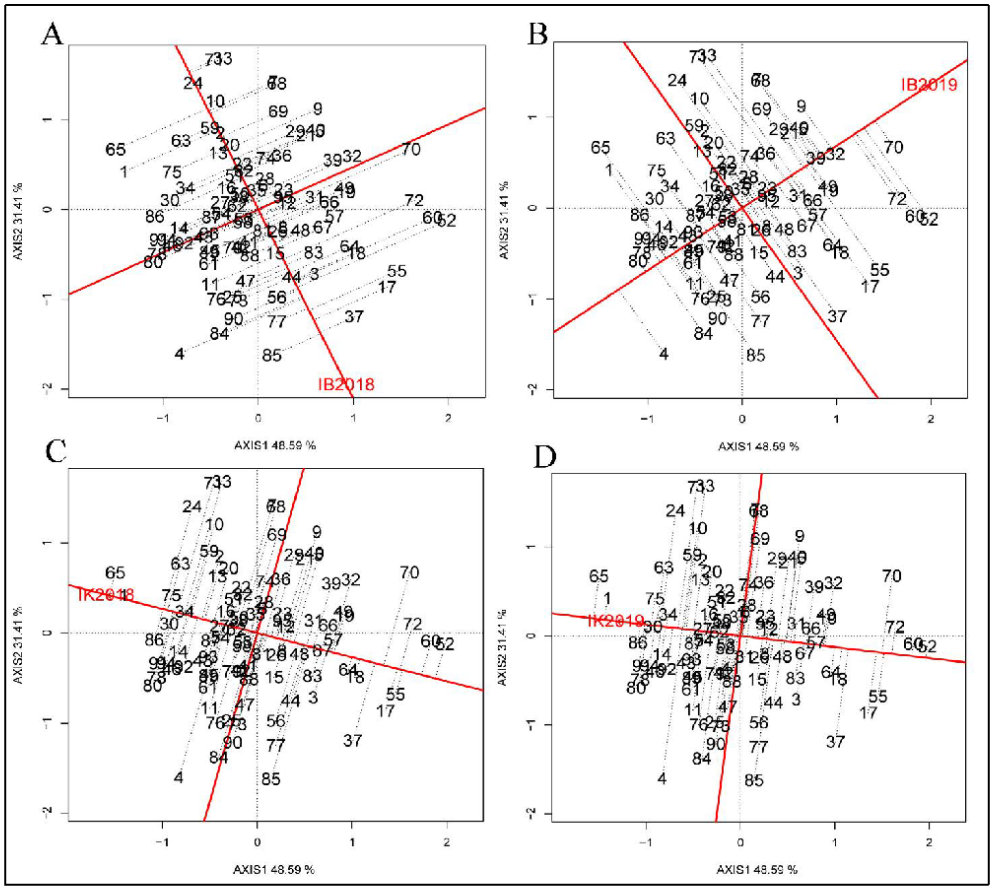

Figure 3. Evaluation of the performances of accessions in the tested environments: (**A**) Ibadan 2018, (B) Ibadan 2019, (C) Ikenne 2018, and (D) Ikenne 2019.

The use of the polygon view of the "which-won-where" biplot is a key component of the GGE, which helps to visualize the interaction patterns between genotypes and environments, to show the presence of crossover GEI, mega-environment differentiation, and specific adaptation [45]. Accessions TVSu-1866, TVSu-2022, TVSu-2017, TVSu-1943, TVSu-1892, TVSu-2060, and TVSu-1557 were all situated at the corners of the polygon (Figure 4), indicating that these accessions were outstanding in terms of their yield in those environments. Accessions/genotypes at the corners of the polygons in a "which-won-where" polygon are the outstanding accessions/genotype in that environment [45]. Among these accessions, TVSu-1943 was the highest-yielding accession in all test environments. Some accessions such as TVSu-1706, TVSu-2018, TVSu-1785, TVSu-1895, and TVSu-1951 were located close to the center of the GGE biplot. This indicates that they showed a stable performance across the test sites [45]. The result in Figure 4 indicates three mega-environments: IB2019, IB2018, and IK2018 and IK2019 together forming the third environment.

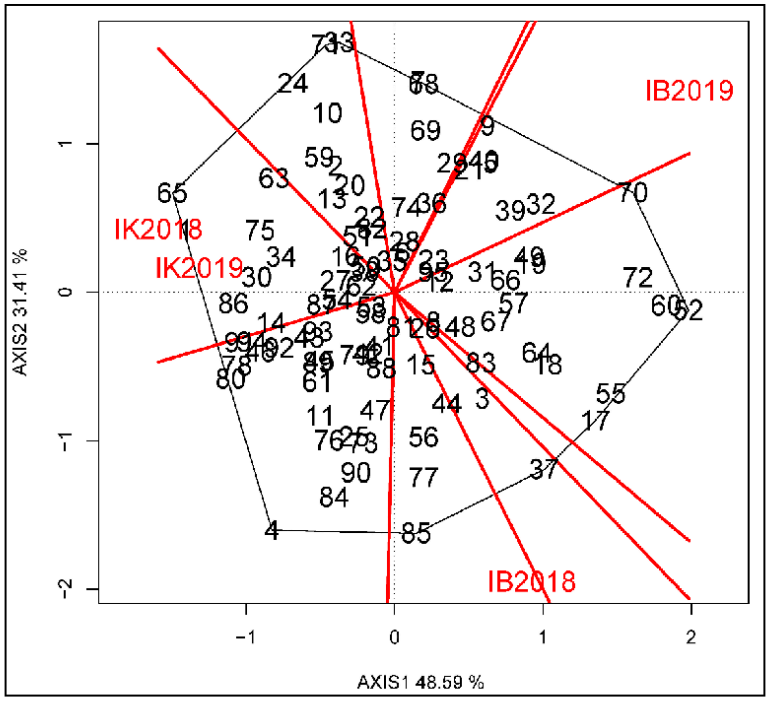

Figure 4. Which-won-where analysis of the accessions. Identifying the best accessions suitable for each test environment.

Figure 5 shows the comparison plot for environments and accessions. Ideal environments and accessions are those which are near or at the center of the concentric circle. Therefore, in this study, the plot in Figure 5A reflected that IB2019 is the closest to being an ideal environment, while TVSu-2020 and TVSu-1649 are the ideal accessions, as shown by their positions (Figure 5B), followed by accessions TVSu-2021, TVSu-1664, TVSu-1866, and TVSu-2025. Accessions close to the ideal accessions are also said to be good. However, accessions TVSu-1557, TVSu-2060, TVSu-2056, and TVSu-2042 are the worst accessions, as they are located far from the concentric circle. In the relationship among the environments, all the environments are positively correlated to one another, but IK2018 and IK2019 are more highly correlated with one another than all the other environments (Figure 5C).

High nutrient uptake, ability to compete favorably with weeds, and yield improvement are some of the prerequisites for developing high-yield crops. The significant differences and the high coefficient of variation observed (39%) (Table 3) indicate the existence of variability in the selected population that can be exploited for an improved breeding program. Variability in traits aid in the trait-assisted selection of best lines for improvement [46,47]. This result is supported by the studies of Olukolu et al. [48] and Gbaguidi et al. [49]. Various studies have shown the GEI effect on several crops such as cassava [50], rice [51,52], sweet potato [53], and sorghum [54]. Yan and Kang [19] stated that the number of genotypes and environments determines the extent of environmental variation. However, according to Aremu et al. [55], the environment is always the dominant source of variation, and it must be prioritized in plant breeding.

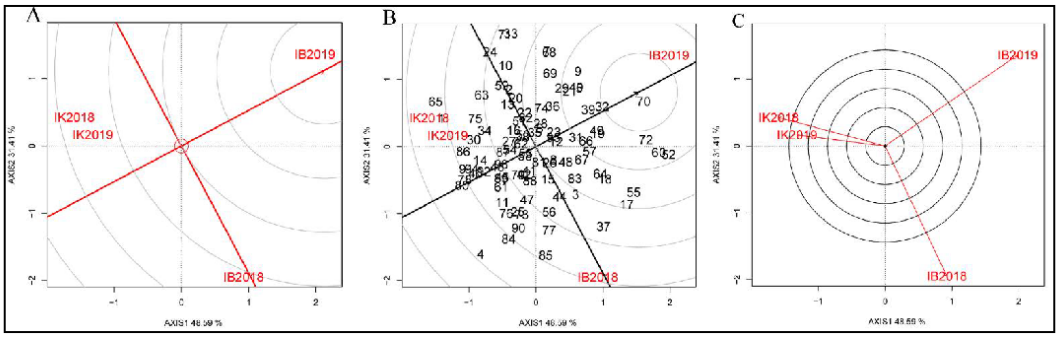

Figure 5. Ranking environments and genotypes based on both mean and stability relative to an ideal genotype. (**A**) Ranking environments with respect to an ideal environment. (B) Ranking genotypes with respect to an ideal genotype. (C) Relationship among tested environments.

Stability and adaptability are important factors in determining the production efficiency of plant varieties. An accession can be good if it has high grain production and potential for improving its production even in varying environments [56]. Thus, adaptability and stability evaluation are important in improving crop production. GGE biplot is effective in analyzing stability and adaptability in MET [57].

Selection of test environments should consider the discriminating ability due to genotype differences and representative ability to represent target environments [37,58]. In the present study, IB2019 has a longer vector and the smallest angle with an ideal environment, so it is identified in this study as a perfect test environment in terms of being more discriminating and most representative of the overall test environments.

4. Conclusions

First, the number of environments and agro-ecological zones can be increased to allow for greater diversity in test locations. This will aid in drawing more accurate conclusions from the outputs. Second, because there have been few or no reports of MET in BGN yield using GGE biplot, many accessions can be used for research. Finally, this study identified genotypes that are uniquely adapted to each environment by observing how different genotypes performed in each of these locations. This knowledge will enable breeders to advise farmers appropriately on which accession to use where, provided that the various accessions meet the end-user quality preferences. Thus, stability analyses aided in the discovery of unique genotypes for all environments studied, as well as a stable genotype that can be cultivated in all the environments studied and in areas with similar characteristics to the test environments. This can be used as a preliminary study for future breeding programs.

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CHAPTER 5: ARTICLE 4

Nutrient and anti-nutrient factors of Bambara groundnut (*Vigna subterranea* (l.) Verdc.) seeds

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Abstract

Food and nutrition security is one of the main goals of sustainable development in this era of climate change. Accessibility to food crops that can almost provide the required nutrients and minerals is an advantage. A balanced diet that is sourced in the right proportions of minerals and nutrients helps maintain proper body function and growth. Bambara groundnut (BGN) fits the bill when it comes to an acceptable level of nutrient and mineral composition. BGN is a balanced food that can help eradicate food and nutritional insecurity if it is incorporated into the major food system. However, there is a large degree of variation in nutrient composition and antinutritional factors among BGN accessions. Here we show the degree of variability of nutrient and antinutrient components such as percentage ash, moisture, protein, fat, tryptophan, tannin, and phytate contents in seeds of 95 accessions of BGN. Data was subjected to analysis of variance (ANOVA), followed by correlation and principal component analysis. Clustering was done to show the relatedness between the accessions in response to the various traits. A high level of heterogeneity was observed among the accessions for the various traits studied. PC1 and PC2 show 41.2% of the total observed variations. Cluster analysis grouped accessions into four main clusters. This study was able to confirm the high level of diversity in the components of nutrients and antinutrients previously reported in BGN. The results of this study are expected to aid in identifying parent lines for improved breeding programs.

Keywords: Antinutritional factors, Bambara groundnut, Food composition, Food security, Multivariate analysis, Proximate analysis

5.1 Introduction

The world population is predicted to exceed 9 billion by 2050 (McKenzie and Williams, 2015). This, along with the impact of climate change, the unavailability of agricultural land, and the reliance on limited species of crop, has hampered the global achievement of food and nutrition security. In sub-Saharan Africa and parts of Asia, the effect of climate change is erratic. Flooding, drought, and increase in temperature are experienced in these areas (Plänitz, 2019; Tesfaye et al., 2018). Farmlands are affected due to environmental impacts from climate change effects such as temperature, humidity, rainfall pattern, and changes in light intensity and duration. Erratic yield and in some cases total loss have been the result (van der Geest et al., 2019). Although these cannot be attributed to climate change, biotic factors such as pests, rodents, birds, and other small mammals have their fair share of recorded losses. Food being produced is merely meeting the demands of the ever-increasing population. Food and nutrition insecurity is taking its toll on the less developed nations. According to FAO, we will need to produce 60% more food if we are to sustain the population by 2050 (www.fao.org/sustainability/en/).

The nutritional and mineral components are vital to the survival of humans and livestock. Lack of adequate amount of which can result in various fatal illnesses. The developing world is the most vulnerable to nutrient and mineral deficiencies. Nutrients are necessary for proper development, and adequate supply is present in most crops, but most of these crops are significantly underutilized in the world food system. Most of these crops are an important part of their immediate local food system. One of these food crops is Bambara groundnut (BGN). Due to its high protein content (9.60-40.0%) (Mohammed, 2014; Oyeyinka et al., 2019) and good balance of essential amino acids (Yao et al., 2015), it is regarded as a complete food (Adebayo-Oyetoro et al., 2017; Oyeyinka and Oyeyinka, 2017). Hence, it can be a good alternative to meat in terms of protein source. In terms

of nutrient components, it competes well with the major food crops, with very few boasting higher nutritional and mineral contents (Babalola et al., 2017). The high fiber and protein content also makes it an ideal component for animal feed. With all the promising attributes of BGN, it is reported to contain some antinutrient components such as phytates and tannins (Halimi et al., 2019; Popova and Mihaylova, 2019). These are not good for consumption in large quantities.

Variations in nutrient compositions occupy a unique role in achieving food and nutritional security in the developing world. Diversities in nutrient and mineral composition enhance the reputation of BGN as a complete food. However, the presence of antinutrient factors has a significant effect on the selection and use of some of the accessions. The high level of these factors in these accessions should be taken seriously, as antinutrient factors have been reported to affect the bioavailability of nutrients and minerals by chelating with the required minerals (Akkad et al., 2019) thereby limiting the concentration of nutrients available for use. However, the situation can be taken care of by fermentation of the seeds and other processing methods as reported in some studies (Belmiro et al., 2020; Nwadi et al., 2020; Qaku et al., 2020).

In terms of trait improvement, no known varieties of BGN have been developed for improvement. The major hindrance to trait development includes a lack of a complete and well-annotated reference genome for the crop. However, other approaches can be used to improve the crop based on its nutrient and antinutrient composition, such as cooking processes (Nwadi et al., 2020). One of such approaches is the use of a genome-wide association study (GWAS) approach. GWAS has been used to develop small nucleotide polymorphism (SNP) markers for regions of simple and complex traits in crops (Gyawali et al., 2019; Kainer et al., 2019). Due to the success of this technology in other crops (Kainer et al., 2019; Sheoran et al., 2019), it will be of great interest if BGN can also benefit to improve its nutrient composition especially, region mapping the

antinutrient components. Locating the region mapping the antinutrient traits can aid the removal of these traits using advanced technologies like gene editing, thereby producing a safe and nutrient-enriched complete food crop.

How well can BGN improve nutritional security, especially in sub-Saharan Africa? Most people, especially in the developed world prefer the major crops for their source of nutrients and global acceptability, however, most underutilized crops reportedly contain enough of these nutrients as is found in the major crops. Therefore, in this study, we will estimate the amount of nutrients and antinutrient components in selected accessions of BGN. Having this information will be key for nutrient-improvement programs in BGN which will facilitate its incorporation into the food system in ensuring food and nutrition security in sub-Saharan Africa and the world at large.

5.2 Materials and methods

5.2.1 Source and Preparation of Bambara Groundnut Seed Materials

Ninety-five accessions of BGN seeds obtained from the genetic resource center of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were selected. The seeds from each accession were milled into powder in the laboratory using a grinder (Link, 1995). 20g of milled seeds were used for the analysis.

5.2.2 Nutrient components

5.2.2.1 Moisture content

Three grams of the milled sample was weighed out in duplicates and placed into moisture canisters. The samples were then placed in an oven (Memmert, GmbH, Model-30-750) for 16hrs at a temperature of 105 °C. The weight was taken every hour until it became constant. Percentage moisture content was calculated according to the formula:

$$\%Moisture\ content\ \left(\frac{wt}{wt}\right) = \frac{[wt\ of\ wet\ sample - wt\ of\ dry\ sample] * 100}{wt\ of\ wet\ sample}$$

Where wt = weight

5.2.2.2 Protein content

The protein content was estimated from the nitrogen content which was determined using the micro Kjeldahl N distillation method and multiplied by 6.25, the universally accepted factor for protein content estimation. Then 0.2g of each of the powdered sample was weighed into digestion tubes of a Foss Tecator^{TM} Digestor, and 4ml each of concentrated 98% H_2SO_4 and H_2O_2 were added. The copper tablet was used as the catalyst. The tubes were heated to 420 °C at the digestion block until a clear solution was obtained. The resultant was distilled using Kjectec 2200 distillation apparatus and the automated Titer equipment was used for titration. The percentage of protein content displayed on the screen was recorded (AOAC, 2005).

5.2.2.3 Determination of fat content

The procedure in Official Methods of Analysis (AOAC, 1990) was followed using Foss Soxtec 2055 fat extraction and auto-analyzer equipment. Three grams of powdered sample was put in a petroleum ether extracting tube and heated between 100 and 145 °C; the reflux continued until the oil in the samples was leached out of the paper into a cup, leaving the water to evaporate. The oil was allowed to cool down and the volume was obtained as shown in the formula.

From the differential weight after evaporation as compared with the initial weight before evaporation occurred.

$$\%\,Fat/_{Oil} = \frac{[(W3-W2)*100]}{W1}$$

Where:

W3 = weight of the tube with the extracted oil,

W2 = weight of the empty tube, and

W1 = weight of the sample.

5.2.2.4 Determination of ash content

The procedure for ash content determination involves weighing 2g of the powdered sample into a crucible with the weight already known. It was oven-dried for 4hrs at 105 °C then put in a muffle furnace (Vulcan, Model-3-1750) at 550°C until white ash was obtained (AOAC, 2005). The samples were cooled in a bench-top desiccator and reweighed to estimate the amount of ash. Percentage ash content was calculated using the formula;

$$\%Ash = \frac{[(weight\ of\ crucible + ash) - (weight\ of\ empty\ crucible)] * 100}{sample\ weight}$$

5.2.2.5 Determination of tryptophan content

The determination of the amino acid tryptophan was done as a separate analysis, due to its differing hydrolysis condition. The samples (400 mg) were hydrolyzed under alkaline conditions with a saturated barium hydroxide solution heated to 110 °C for 20 hrs. The hydrolysate was analyzed by reverse-phase liquid chromatography with UV detection at 285 nm, using a Waters Breeze HPLC with Empower software (Waters, Millipore Corp., Milford, MA) (Bertacco et al., 1992).

5.2.2.6 Determination of carbohydrate content

This was derived by subtracting the result obtained from ash, protein, moisture content, and fat, from 100, i.e., 100 – (ash + protein + moisture content + fat) (Drapala et al., 2016).

5.2.3 Anti-nutritional components

5.2.3.1 Determination of phytic acid

This was analyzed with 1 g of the sample. Extraction and precipitation were done according to the method of Wheeler and Ferrel (1971). The phytic content of the samples was then determined by a 4:6 Fe/P atomic ratio.

5.2.3.2 Determination of tannins

This was done determined according to the method of Adegunwa et al. (2011). A mixture of 0.5g of the sample in 50 ml distilled water was allowed to stand for 30 mins at 28 °C and filtered using Whatman No. 42 filter paper. After dispensing about 2 ml of the extract into a 50 ml volumetric flask, the standard tannin solution (2 ml) and 2 ml of distilled water were put in separate volumetric flasks to serve as standards. Folins reagent and 2.5 ml of saturated NA_2CO_3 solution were added to each flask. The contents of each flask were made up to 50 ml with distilled water and incubated at 28 °C for 90 min. The absorbance was measured at 260 nm on a spectrophotometer (ThermoFisher Scientific, Model, G10 UV-VIS). The reagent blank was used to calibrate the instrument at zero.

5.2.4 Statistical Analysis

All analysis was done using the R statistical package (R Core Team, 2019). Descriptive and summary statistics were computed using the *stat.dev* function in the pastec package. One-way analysis of variance (ANOVA) was performed using the *lmer* function to determine significant differences between the means of the nutrients and antinutrient components. Fischer's least significant difference (F-LSD) at a probability level of 5% was used to separate the means that were significantly different. Principal component analysis was done using the subsequent cluster analysis from the *fviz* function in the factominer package, and correlation coefficients to view the groupings and relatedness among these collections of accessions on the different traits.

5.3 Results

5.3.1 Descriptive statistics and ANOVA analysis

Descriptive statistics showed a significant coefficient of variations (CV>20) in the contents of ash, moisture, tryptophan, and tannin (Table 5.1). The range of 50.6-69.3, 0.76-6.39, 2.88-13.55, 12.51-

26.72, 3.19-9.88, 0.09-0.47, 0.08-0.42, and 3.17-7.42 was recorded for carbohydrate, ash, moisture, protein, fat, tryptophan, tannin, and phytate contents respectively. Among the nutrient components, carbohydrate had the highest range but the lowest variation while phytate had the highest range between the two antinutrient components as well as the lowest variation. In the ANOVA result (Table 5.2), all components were highly significant ($p<0.0001$). The resulting means separation showed a high level of heterogeneity among the accessions for the traits, especially for ash, moisture, phytate, and tryptophan contents (Table 5.3).

Table 5.1. Descriptive statistics of the nutrient and anti-nutrient compositions.

Traits	Mean	Std Error	Std Dev	C V	Min	Max	Range
Carbohydrate (%)	58.34	0.30	4.12	0.07	50.6	69.3	18.7
Ash (%)	3.82	0.07	0.91	23.78	0.76	6.39	5.63
MC (%)	8.86	0.19	2.62	29.50	2.88	13.55	10.67
Protein (%)	22.43	0.16	2.12	9.45	12.51	26.72	14.21
Fat (%)	6.49	0.09	1.20	18.51	3.19	9.88	6.69
Tryptophan	0.21	0.00	0.06	30.34	0.09	0.47	0.38
Tannin (%)	0.20	0.01	0.08	40.45	0.08	0.42	0.34
Phytate (mg/100g)	5.61	0.07	0.95	16.94	3.24	8.32	5.08

†Std Error: standard error. Std Dev: standard deviation. CV: coefficient of variation. Min: minimum. Max: maximum.

Table 5.2 ANOVA table for the trait's responses.

Traits	Variation source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	LSD
Carbohydrate	Accns	94	3198.7	34.029	244.19	< 2.2e-16 ***	0.74
	Residuals	95	13.2	0.139			
Ash	Accns	94	150.538	1.60146	60.483	< 2.2e-16 ***	0.32
	Residuals	95	2.515	0.02648			
Fat	Accns	94	266.28	2.83279	145.43	< 2.2e-16 ***	0.28
	Residuals	95	1.85	0.01948			
Moisture content	Accns	94	1260.6	13.4107	247.2	< 2.2e-16 ***	0.46
	Residuals	95	5.15	0.0543			
Phytate	Accns	94	185.56	1.97404	72.88	< 2.2e-16 ***	0.33

	Residuals	95	2.573	0.02709			
Protein	Accns	94	831.91	8.8501	199.33	< 2.2e-16 ***	0.42
	Residuals	95	4.22	0.0444			
Tannin	Accns	94	1.2136 7	0.0129	72.365	< 2.2e-16 ***	0.03
	Residuals	95	0.0169 5	0.00017			
Tryptophan	Accns	94	1.376	0.01463	8.454	< 2.2e-16 ***	0.08
	Residuals	95	0.1645	0.00173			

† LSD: least significant difference. ***Highly significant at $p < 0.0001$.

Table 5.3. Means±standard deviation of nutrient and anti-nutrient components in each accession.

Accessions	Nutrient components						Anti-nutrient components	
	CHO	Ash	MC	Protein	Fat	Tryptophan	Tannin	Phytate
TVSu-1470	59.32±0.56^{t-w}	4.16±0.00^{k-t}	7.73±0.35^{BCD}	23.22±0.01^{q-u}	5.59±0.90^{xyz}	0.14±0.00^{n-s}	0.13±0.00^{G-L}	8.32±0.00^{a}
TVSu-1538	59.72±0.03^{r-u}	3.36±0.12^{E-I}	7.55±0.07^{CD}	22.21±0.02^{BCD}	7.16±0.01^{lm}	0.20±0.00^{i-p}	0.09±0.00^{OP}	5.41±0.03^{y-D}
TVSu-1547	59.51±0.06^{s-v}	2.17±0.00^{Q}	7.96±0.01^{A-D}	23.22±0.00^{q-u}	7.16±0.02^{lm}	0.18±0.00^{j-r}	0.12±0.00^{I-N}	5.12±0.00^{D-H}
TVSu-1557	60.32±0.04^{qr}	2.13±0.06^{QR}	8.24±0.08^{w-A}	23.11±0.03^{r-v}	6.21±0.01^{s-v}	0.18±0.00^{j-r}	0.13±0.00^{G-L}	5.65±0.07^{v-A}
TVSu-1574	57.70±0.03^{CDE}	6.16±0.00^{ab}	8.13±0.00^{y-B}	23.06±0.02^{r-v}	4.96±0.00^{B}	0.14±0.00^{n-s}	0.12±0.01^{J-O}	4.49±0.03^{JK}
TVSu-1589	62.80±0.02^{g}	3.18±0.03^{G-L}	8.48±0.16^{u-y}	19.92±0.12^{MN}	5.63±0.03^{xy}	0.13±0.01^{p-s}	0.13±0.01^{H-M}	5.38±0.07^{z-E}
TVSu-1649	60.33±0.18^{qr}	4.20±0.22^{j-s}	8.17±0.03^{y-B}	22.49±0.04^{y-B}	4.83±0.02^{C}	0.16±0.01^{l-s}	0.11±0.00^{K-P}	4.35±0.10^{JKL}
TVSu-1663	61.90±0.37^{ijk}	4.12±0.30^{l-t}	8.10±0.04^{y-B}	21.10±0.01^{GHI}	4.80±0.00^{C}	0.16±0.01^{l-s}	0.19±0.01^{w-A}	4.05±0.07^{L-O}
TVSu-1664	58.71±0.12^{w-A}	3.99±0.05^{p-w}	8.15±0.06^{y-B}	22.96±0.01^{t-w}	6.20±0.02^{s-v}	0.18±0.01^{j-r}	0.17±0.01^{z-E}	3.78±0.07^{NO}
TVSu-1680	66.35±0.49^{c}	3.86±0.19^{t-A}	8.16±0.10^{y-B}	16.28±0.06^{Q}	5.36±0.00^{yz}	0.17±0.01^{k-s}	0.17±0.00^{y-D}	4.18±0.06^{KLM}
TVSu-1701	62.58±0.12^{ghi}	3.55±0.13^{A-F}	8.27±0.01^{w-A}	22.40±0.01^{z-C}	3.21±0.02^{D}	0.19±0.01^{j-q}	0.16±0.00^{A-F}	5.96±0.04^{p-v}
TVSu-1706	62.94±0.08^{g}	3.45±0.02^{B-G}	8.32±0.05^{w-A}	19.68±0.00^{NO}	5.61±0.02^{xyz}	0.17±0.01^{k-s}	0.19±0.01^{w-A}	4.62±0.04^{IJ}
TVSu-1724	68.76±0.00^{a}	3.05±0.05^{H-N}	8.49±0.03^{u-y}	12.52±0.00$_{R}$	7.19±0.02^{im}	0.24±0.01^{f-l}	0.19±0.00^{v-z}	5.19±0.00^{C-G}
TVSu-1733	61.90±0.07^{ijk}	3.74±0.20^{v-B}	8.44±0.05^{v-z}	19.70±0.36^{NO}	6.23±0.02^{r-u}	0.20±0.01^{i-p}	0.27±0.01^{l-o}	3.74±0.03^{O}

TVSu-1739	62.02±0.02^{hij}	2.95±0.03^{L-O}	8.63±0.06^{u-x}	19.88±0.00^{MN}	6.53±0.01^{opq}	0.13±0.00^{p-s}	0.38±0.01^{bcd}	4.35±0.03^{JKL}
TVSu-1740	67.77±0.01^{b}	0.85±0.09^{S}	8.69±0.16^{t-w}	17.50±0.06^{P}	5.21±0.01^{AB}	0.35±0.00^{cde}	0.41±0.01^{a}	3.81±0.10^{NO}
TVSu-1742	61.12±0.60^{l-p}	4.12±0.30^{l-t}	8.01±0.10^{z-C}	20.74±0.00^{IJK}	6.02±0.02^{uvw}	0.41±0.01^{abc}	0.18±0.00^{x-C}	4.17±0.07^{KLM}
TVSu-1745	61.21±0.06^{k-o}	3.01±0.18^{K-O}	9.46±0.27^{opq}	20.94±0.48^{HIJ}	5.39±0.01^{yzA}	0.22±0.02^{g-o}	0.14±0.01^{F-K}	6.13±0.07^{n-s}
TVSu-1758	58.67±0.00^{w-A}	3.60±0.11^{y-F}	8.94±0.08^{r-u}	22.23±0.03^{A-D}	6.57±0.01^{opq}	0.23±0.01^{f-l}	0.12±0.01^{J-O}	5.41±0.04^{y-D}
TVSu-1763	61.64±1.04^{jkl}	1.84±0.10^{R}	7.57±0.80^{CD}	23.38±0.02^{o-s}	5.58±0.02^{xyz}	0.22±0.01^{g-o}	0.30±0.01^{h-k}	6.93±0.06^{d-g}
TVSu-1764	59.07±0.21^{u-y}	3.37±0.15^{E-H}	8.53±0.04^{u-y}	22.85±0.03^{u-y}	6.19±0.01^{s-v}	0.23±0.00^{f-l}	0.21±0.00^{t-w}	6.68±0.03^{g-j}
TVSu-1765	59.93±0.99^{rst}	3.19±0.16^{G-L}	9.45±0.16^{opq}	21.83±0.02^{DEF}	5.61±0.05^{xyz}	0.22±0.00^{g-n}	0.27±0.01^{m-p}	5.49±0.03^{y-C}
TVSu-1785	60.89±0.14^{m-q}	3.19±0.02^{G-L}	9.27±0.10^{p-s}	21.83±0.05^{DEF}	4.83±0.02^{C}	0.21±0.01^{h-p}	0.38±0.01^{abc}	6.26±0.07^{l-p}
TVSu-1787	61.48±0.02^{j-n}	3.04±0.10^{J-N}	9.44±0.00^{opq}	19.85±0.24^{N}	6.21±0.01^{s-v}	0.14±0.00^{n-s}	0.40±0.01^{ab}	5.85±0.00^{s-x}
TVSu-1823	57.73±0.02^{CDE}	3.90±0.15^{s-y}	9.13±0.03^{q-t}	23.46±0.02^{n-r}	5.79±0.00^{wx}	0.19±0.01^{j-q}	0.18±0.00^{x-C}	6.37±0.05^{j-n}
TVSu-1836	60.68±0.05^{opq}	3.42±0.04^{C-G}	9.57±0.22^{opq}	19.94±0.11^{MN}	6.40±0.03^{qrs}	0.20±0.01^{i-p}	0.35±0.01^{def}	5.11±0.06^{D-H}
TVSu-1839	57.00±0.02^{EFG}	4.25±0.01^{i-q}	9.34±0.11^{o-r}	23.19±0.01^{r-u}	6.23±0.02^{r-u}	0.19±0.01^{j-q}	0.33±0.00^{efg}	6.15±0.03^{n-s}
TVSu-1843	59.15±0.04^{u-x}	3.68±0.08^{w-E}	8.37±0.02^{w-A}	22.04±0.02^{CDE}	6.77±0.01^{no}	0.23±0.00^{f-l}	0.35±0.00^{ef}	5.48±0.02^{y-C}
TVSu-1850	57.34±0.01^{DEF}	2.50±0.02^{P}	9.13±0.08^{q-t}	24.64±0.04^{d-g}	6.40±0.02^{qrs}	0.21±0.01^{h-p}	0.15±0.00^{D-I}	4.58±0.06^{IJ}
TVSu-1851	55.33±0.27^{M-R}	3.36±0.20^{E-I}	9.64±0.02^{op}	24.92±0.04^{cd}	6.76±0.01^{no}	0.25±0.01^{f-k}	0.12±0.01^{J-O}	3.90±0.06^{MNO}

TVSu-1859	56.48±0.43^{G-J}	3.99±0.09^{p-w}	9.33±0.21^{o-r}	24.22±0.02^{h-k}	5.99±0.01^{uvw}	0.24±0.01^{f-l}	0.12±0.01^{J-O}	4.16±0.07^{KLM}
TVSu-1863	56.08±0.51^{I-L}	3.44±0.03^{B-G}	10.73±0.08^{c-k}	24.75±0.03^{def}	5.01±0.44^{BC}	0.18±0.00^{j-r}	0.14±0.01^{E-J}	5.24±0.06^{B-F}
TVSu-1866	58.80±0.50^{v-z}	3.40±0.20^{D-G}	9.75±0.08^{no}	21.46±0.01^{FG}	6.60±0.24^{opq}	0.20±0.01^{i-p}	0.17±0.01^{z-E}	5.37±0.06^{A-E}
TVSu-1868	55.71±0.56^{K-P}	3.31±0.07^{F-K}	9.43±0.08^{opq}	25.42±0.21^{b}	6.14±0.18^{s-v}	0.22±0.01^{g-o}	0.28±0.01^{k-n}	4.63±0.00^{IJ}
TVSu-1874	56.38±0.70^{G-K}	3.15±0.18^{G-M}	8.20±0.08^{x-A}	23.68±0.23^{m-p}	8.60±0.17^{c}	0.24±0.01^{f-l}	0.24±0.01^{p-s}	5.06±0.14^{E-H}
TVSu-1879	55.10±0.13^{O-T}	3.04±0.14^{I-N}	10.33±0.01^{i-m}	25.60±0.01^{b}	5.94±0.06^{vw}	0.20±0.01^{i-p}	0.25±0.01^{o-r}	6.24±0.07^{l-q}
TVSu-1892	57.64±0.06^{C-E}	3.37±0.16^{E-H}	10.85±0.03^{c-h}	20.79±0.24^{IJK}	7.36±0.01^{kl}	0.16±0.01^{l-s}	0.29±0.00^{i-m}	6.32±0.07^{k-o}
TVSu-1895	60.44±0.51^{p-r}	2.78±0.13^{N-P}	10.63±0.03^{d-l}	20.96±0.47^{HI}	5.20±0.02^{AB}	0.14±0.01^{o-s}	0.14±0.00^{E-J}	5.09±0.10^{D-H}
TVSu-1898	52.59±0.30^{Y}	4.51±0.13^{f-j}	10.93±0.00^{c-f}	22.59±0.05^{w-B}	9.40±0.04^{b}	0.11±0.01^{qr}	0.16±0.01^{B-G}	6.19±0.07^{m-r}
TVSu-1899	54.41±0.25^{T-W}	3.75±0.12^{v-B}	10.87±0.10^{c-h}	22.42±0.03^{z-C}	8.55±0.02^{c}	0.14±0.01^{o-s}	0.18±0.00^{x-C}	5.46±0.07^{y-C}
TVSu-1905	54.53±0.80^{S-W}	4.56±0.11^{e-i}	10.80±0.10^{c-h}	20.29±0.50^{L} M	9.83±0.05^{a}	0.16±0.01^{l-s}	0.23±0.01^{rst}	7.03±0.03^{c-f}
TVSu-1912	50.85±0.33^{1}	4.75±0.06^{d-g}	10.74±0.13^{c-k}	24.30±0.01^{g-j}	9.37±0.03^{b}	0.18±0.01^{j-r}	0.28±0.00^{j-n}	6.79±0.07^{e-i}
TVSu-1915	51.23±0.03^{Z1}	4.37±0.02^{h-n}	10.62±0.04^{e-m}	24.20±0.00^{h-k}	9.60±0.04^{ab}	0.20±0.01^{i-p}	0.30±0.01^{i-l}	6.06±0.06^{n-s}
TVSu-1918	57.77±0.23^{BCD}	3.60±0.11^{y-F}	10.63±0.08^{d-l}	21.78±0.01^{EF}	6.23±0.02^{r-u}	0.21±0.01^{h-p}	0.35±0.00^{ef}	5.12±0.07^{D-H}
TVSu-1920	53.29±0.35^{XY}	4.94±0.21^{d}	10.86±0.00^{c-h}	22.53±0.03^{x-B}	8.39±0.01^{cd}	0.23±0.01^{g-m}	0.17±0.01^{z-E}	6.73±0.07^{f-i}
TVSu-1921	54.65±0.01^{R-W}	4.44±0.14^{g-l}	10.63±0.20^{d-l}	24.30±0.05^{g-j}	5.99±0.01^{uvw}	0.17±0.00^{k-s}	0.27±0.01^{m-p}	5.52±0.07^{y-B}

TVSu-1923	55.33±0.13^{M-R}	5.99±0.02^{b}	10.61±0.06^{e-m}	24.68±0.00^{d-g}	3.40±0.00^{D}	0.47±0.00^{a}	0.31±0.01^{ghi}	6.59±0.07^{h-k}
TVSu-1930	55.22±0.12^{N-S}	4.29±0.11^{h-p}	10.88±0.08^{c-g}	23.41±0.04^{o-s}	6.21±0.01^{s-v}	0.23±0.01^{f-l}	0.36±0.01^{cde}	5.99±0.07^{p-u}
TVSu-1937	56.87±0.08^{FGH}	4.24±0.02^{j-r}	10.79±0.06^{c-j}	21.32±0.01^{GH}	6.80±0.00^{no}	0.25±0.01^{f-k}	0.25±0.01^{o-r}	5.52±0.07^{y-C}
TVSu-1939	54.95±0.04^{Q-U}	4.49±0.25^{g-j}	10.32±0.28^{klm}	22.24±0.01^{A-D}	8.01±0.04^{ef}	0.28±0.01^{e-i}	0.31±0.01^{g-j}	6.22±0.03^{m-q}
TVSu-1941	51.24±0.22^{Z1}	6.33±0.06^{a}	10.33±0.00^{j-m}	24.46±0.10^{e-i}	7.66±0.01^{hij}	0.10±0.00^{s}	0.22±0.01^{s-v}	5.25±0.07^{B-F}
TVSu-1943	53.29±0.04^{XY}	4.12±0.02^{l-t}	10.74±0.05^{c-k}	23.77±0.01^{l-o}	8.09±0.11^{ef}	0.39±0.04^{bcd}	0.33±0.01^{efg}	4.18±0.06^{KLM}
TVSu-1945	51.69±0.21^{Z}	4.14±0.10^{l-t}	10.73±0.03^{c-k}	25.21±0.01^{bc}	8.24±0.26^{de}	0.29±0.00^{efg}	0.17±0.01^{z-E}	6.72±0.07^{f-i}
TVSu-1951	55.27±0.25^{N-R}	4.13±0.13^{l-t}	10.79±0.02^{c-i}	22.23±0.02^{A-D}	7.59±0.01^{ijk}	0.28±0.01^{e-i}	0.19±0.01^{w-A}	7.32±0.07^{bc}
TVSu-1952	53.22±0.30^{XY}	3.33±0.26^{F-J}	10.72±0.00^{c-k}	24.37±0.01^{f-j}	8.38±0.03^{cd}	0.31±0.00^{def}	0.25±0.01^{o-r}	6.56±0.02^{h-l}
TVSu-1956	53.91±0.10^{WX}	4.30±0.20^{h-p}	10.52±0.17^{f-m}	23.88±0.02^{k-n}	7.40±0.02^{jkl}	0.29±0.01^{efg}	0.16±0.01^{B-G}	6.75±0.02^{f-i}
TVSu-1957	54.43±0.05^{T-W}	4.09±0.05^{m-t}	10.58±0.00^{e-m}	23.33±0.01^{p-t}	7.58±0.01^{ijk}	0.46±0.01^{ab}	0.24±0.00^{qrs}	6.48±0.02^{i-m}
TVSu-1959	52.97±0.39^{Y}	4.40±0.05^{h-n}	13.43±0.13^{a}	22.21±0.07^{BCD}	7.01±0.04^{mn}	0.13±0.01^{p-s}	0.19±0.01^{w-A}	6.26±0.06^{l-p}
TVSu-1962	55.82±0.23^{J-O}	3.93±0.17^{r-x}	10.72±0.04^{c-k}	22.77±0.04^{v-z}	6.78±0.01^{no}	0.15±0.01^{m-s}	0.21±0.00^{t-w}	3.98±0.00^{MNO}
TVSu-1964	57.93±0.16^{BCD}	0.85±0.09^{S}	10.85±0.01^{c-h}	22.96±0.02^{t-w}	7.43±0.02^{jkl}	0.17±0.01^{k-s}	0.33±0.01^{fgh}	6.12±0.02^{n-s}
TVSu-1972	54.74±0.16^{R-V}	4.38±0.13^{h-n}	11.08±0.04^{cd}	23.41±0.02^{o-s}	6.40±0.01^{qrs}	0.19±0.01^{j-q}	0.11±0.00^{K-P}	5.72±0.08^{t-y}
TVSu-1979	54.23±0.04^{UVW}	2.85±0.03^{MNO}	10.81±0.03^{c-h}	25.56±0.00^{b}	6.56±0.02^{opq}	0.22±0.01^{g-o}	0.30±0.00^{h-k}	5.58±0.01^{x-A}

TVSu-2000	55.06±0.11^{P-T}	4.85±0.06^{de}	11.13±0.03^{c}	24.02±0.04^{j-m}	4.95±0.06^{BC}	0.10±0.01^{rs}	0.14±0.01^{F-K}	6.36±0.23^{j-n}
TVSu-2003	56.06±1.46^{I-M}	2.69±0.00^{OP}	12.33±1.03^{b}	22.43±0.00^{z-C}	6.49±0.00^{pqr}	0.29±0.03^{efg}	0.20±0.00^{t-x}	5.61±0.00^{w-A}
TVSu-2017	55.91±0.00^{I-N}	4.23±0.02^{j-r}	10.94±0.02^{c-f}	22.43±0.00^{z-C}	6.49±0.00^{pqr}	0.30±0.02^{efg}	0.20±0.00^{t-x}	4.85±0.07^{HI}
TVSu-2018	60.76±0.01^{n-q}	2.92±0.02^{L-O}	8.86±0.00^{s-v}	21.08±0.01^{GHI}	6.39±0.02^{qrs}	0.39±0.12^{bcd}	0.09±0.01^{p}	5.06±0.06^{E-H}
TVSu-2019	54.40±0.08^{T-W}	4.34±0.03^{h-o}	4.34±0.03^{c-i}	24.84±0.03^{cde}	5.64±0.06^{xy}	0.26±0.05^{f-j}	0.23±0.01^{rst}	7.44±0.38^{b}
TVSu-2020	54.13±1.02^{VW}	4.51±0.13^{f-j}	10.85±0.04^{c-h}	22.61±0.44^{w-B}	7.91±0.11^{fg}	0.29±0.23^{e-h}	0.23±0.01^{rst}	6.00±0.02^{o-t}
TVSu-2021	52.77±0.04^{Y}	5.46±0.10^{c}	10.53±0.16^{f-m}	24.84±0.02^{cde}	6.40±0.01^{qrs}	0.14±0.01^{n-s}	0.15±0.00^{D-I}	3.24±0.06^{p}
TVSu-2022	58.06±0.28^{z-D}	4.40±0.10^{h-m}	10.49±0.06^{f-m}	21.04±0.02^{HI}	6.02±0.02^{uvw}	0.16±0.02^{l-s}	0.24±0.00^{qrs}	5.92±0.06^{q-w}
TVSu-2025	58.51±0.35^{x-B}	3.94±0.14^{q-x}	11.00±0.04^{cde}	20.93±0.02^{HIJ}	5.64±0.04^{wy}	0.17±0.01^{k-s}	0.26±0.01^{n-q}	7.11±0.06^{cde}
TVSu-2030	54.68±0.37^{R-V}	5.50±0.12^{c}	10.19±0.15^{lmn}	23.42±0.03^{o-r}	6.22±0.02^{r-u}	0.21±0.02^{h-p}	0.10±0.01^{NOP}	4.90±0.07^{GHI}
TVSu-2031	58.95±0.09^{v-y}	4.20±0.13^{j-s}	7.51±0.05^{D}	23.00±0.00^{s-w}	6.35±0.01^{q-t}	0.17±0.00^{k-s}	0.23±0.01^{rst}	4.10±0.06^{LMN}
TVSu-2032	58.37±0.17^{y-C}	3.66±0.10^{x-E}	10.42±0.03^{h-m}	20.41±0.01^{KL}	7.15±0.02^{lm}	0.47±0.00^{a}	0.18±0.00^{x-C}	7.30±0.13^{bc}
TVSu-2034	56.15±0.74^{H-L}	4.48±0.08^{e-h}	10.43±0.10^{klm}	23.12±0.02^{u-y}	6.35±0.02^{tuv}	0.15±0.08^{m-s}	0.20±0.01^{u-y}	4.97±0.06^{r-x}
TVSu-2038	58.83±0.84^{v-y}	3.88±0.04^{s-z}	10.68±0.04^{c-k}	21.23±0.51^{GH}	5.39±0.01^{yzA}	0.25±0.01^{f-k}	0.10±0.00^{M-P}	5.70±0.07^{t-z}
TVSu-2042	55.87±0.30^{I-N}	4.22±0.11^{j-r}	10.46±0.04^{g-m}	24.12±0.01^{i-l}	5.35±0.04^{zA}	0.28±0.01^{e-i}	0.14±0.01^{F-K}	7.21±0.02^{bcd}
TVSu-2043	55.63±0.20^{L-Q}	4.48±0.11^{g-k}	10.43±0.1^{g-m}	23.12±0.03^{r-v}	6.35±0.04^{q-t}	0.23±0.01^{f-l}	0.20±0.01^{u-y}	4.97±0.09^{FGH}

TVSu-2045	55.63±0.20^{GHI}	4.82±0.15^{def}	10.20±0.00^{lmn}	22.63±0.03^{w-A}	5.80±0.00^{wx}	0.09±0.00^{s}	0.18±0.01^{w-B}	6.79±0.00^{e-i}
TVSu-2046	56.56±0.16^{A-D}	4.38±0.01^{h-n}	10.16±0.12^{mn}	20.95±0.02^{HIJ}	6.55±0.02^{opq}	0.39±0.04^{bcd}	0.09±0.01^{P}	6.63±0.04^{g-k}
TVSu-2048	57.98±0.25^{XY}	4.11±0.11^{m-t}	10.31±0.00^{klm}	26.71±0.01^{a}	5.58±0.00^{xyz}	0.22±0.10^{g-n}	0.16±0.01^{B-G}	5.39±0.06^{z-D}
TVSu-2051	53.30±0.13^{v-y}	4.04±0.12^{o-v}	10.47±0.02^{g-m}	20.54±0.00^{JKL}	6.16±0.02^{s-v}	0.16±0.02^{l-s}	0.17±0.01^{z-E}	5.66±0.07^{u-A}
TVSu-2055	58.81±0.15^{gh}	4.41±0.11^{h-m}	3.04±0.04^{EF}	23.63±0.03^{m-q}	6.18±0.02^{s-v}	0.17±0.01^{k-s}	0.14±0.01^{E-J}	6.36±0.03^{j-n}
TVSu-2056	62.75±0.16^{ij}	4.59±0.04^{e-h}	3.48±0.30^{E}	24.60±0.01^{d-h}	5.38±0.01^{yzA}	0.21±0.02^{h-p}	0.15±0.00^{D-I}	6.63±0.11^{g-k}
TVSu-2060	61.96±0.35^{ef}	3.72±0.01^{v-D}	3.03±0.03^{EF}	22.94±0.05^{t-x}	5.57±0.02^{xyz}	0.17±0.00^{k-s}	0.13±0.01^{H-M}	6.59±0.07^{h-k}
TVSu-2065	64.75±0.13^{a}	3.60±0.02^{y-F}	3.00±0.02^{F}	19.36±0.02^{O}	4.77±0.02^{C}	0.47±0.00^{a}	0.09±0.00^{OP}	6.86±0.07^{e-h}
TVSu-2067	69.29±0.05^{qrs}	4.08±0.02^{n-u}	3.01±0.01^{F}	24.95±0.36^{cd}	7.78±0.02^{ghi}	0.23±0.01^{f-l}	0.11±0.01^{L-P}	5.93±0.06^{q-w}
TVSu-2068	60.19±0.50^{de}	3.73±0.01^{v-C}	3.12±0.07^{EF}	21.24±0.03^{GH}	6.56±0.03^{opq}	0.17±0.11^{k-s}	0.13±0.03^{G-L}	5.39±0.07^{y-D}
TVSu-2071	65.36±0.11^{d}	3.73±0.04^{v-C}	3.05±0.01^{EF}	21.16±0.27^{GHI}	6.58±0.02^{opq}	0.39±0.04^{bcd}	0.17±0.02^{y-D}	6.13±0.00^{n-s}
TVSu-2074	65.49±0.45^{l-p}	4.31±0.02^{h-p}	2.98±0.04^{F}	25.23±0.04^{bc}	6.38±0.02^{qrs}	0.22±0.10^{g-n}	0.18±0.02^{w-B}	6.07±0.07^{n-s}
TVSu-2075	61.12±0.12^{cd}	3.62±0.02^{x-F}	2.97±0.00^{F}	20.95±0.01^{HIJ}	6.71±0.06^{op}	0.16±0.02^{l-s}	0.20±0.03^{u-y}	5.39±0.07^{y-D}
TVSu-2076	65.76±0.08^{f}	4.13±0.03^{l-t}	3.13±0.11^{EF}	21.49±0.13^{FG}	7.19±0.01^{lm}	0.17±0.01^{k-s}	0.23±0.02^{rst}	6.49±0.03^{i-m}
TVSu-2083	64.07±0.08^{j-m}	3.89±0.01^{s-y}	2.99±0.01^{F}	25.21±0.01^{bc}	6.35±0.01^{q-t}	0.02±0.19^{g-m}	0.22±0.04^{r-u}	5.38±0.07^{z-E}
TVSu-2085	61.57±0.01^{g}	3.76±0.05^{u-B}	2.92±0.04^{F}	23.66±0.03^{m-p}	6.77±0.00^{no}	0.35±0.08^{cd}	0.15±0.04^{C-H}	5.66±0.06^{v-A}

TVSu-2086	62.91±0.08^{d}	3.57±0.04$^{z\text{-}F}$	3.07±0.09^{EF}	21.01±0.50^{HI}	6.79±0.01^{no}	0.15±0.01$^{m\text{-}s}$	0.10±0.01^{NOP}	5.89±0.04$^{r\text{-}x}$

Numbers representing means±standard deviation in a column followed by the same letter are not significantly different according to Fischer's least significant difference (LSD) test ($p < 0.05$). MC: Moisture content. Phytate (mg/100g). % Tannin. MC=CHO=Protein=Ash=Fat= (%)

5.3.2 Principal component analysis

When we looked at the variation in nutrient and antinutrient content among the 95 BGN accessions, the first three principal components (PC) with Eigen values ≥1 described around 62% of the variation (Table 5.4). The first, second, and third PCs accounted for 29.74%, 17.55%, and 14.45% of the variations respectively, in the data set under consideration. PC1(Dim.1) was mostly influenced by the following characteristics: CHO (40.97%), moisture content (18.58%), protein (16.87%), ash (10.51%), and fat (9.67%). On the second PC (Dim.2), the variables ash, moisture content, tannin, and protein each accounted for 21.33% of the variations, 16.91% of the variations, 42.66% of the variations, and 11.56% of the variations. Tryptophan (48.70%) and phytate (40.95%) were the most important contributors to the variation described by PC3 (Dim.3).

Table 5.4. Contribution of principal components to the variations of the nutrient and antinutrient components among the BGN accessions.

Traits	Dim.1	Dim.2	Dim.3
CHO	40.97	0.18	0.40
Ash	10.51	21.33	1.65
Fat	9.67	3.99	5.09
Moisture Content	18.58	16.91	2.30
Phytate	2.57	3.34	40.95
Protein	16.87	11.56	0.01
Tannin	0.66	42.66	0.91
Tryptophan	0.18	0.03	48.70
Eigenvalue	2.38	1.40	1.16
Proportion of variance (%)	29.74	17.55	14.45
Cumulative variance (%)	29.74	47.29	61.74

The various links between accessions and attributes are depicted in Fig. 5.1. Those accessions whose vectors are plotted close to the vector for a specific nutrient or antinutrient component are highly correlated to that nutrient or antinutrient component, whereas the length of a vector for an accession will estimate its mean for that specific nutrient or antinutrient component. An accession

that is drawn along with a lengthy vector implies that it has a high mean value for the nutrient or antinutrient that it relates to. In the study of CHO, three accessions were among those identified as being strongly connected with the vector: TVSu-2065, TVSu-2071, and TVSu-2086. A few accessions associated with ash were identified, including TVSu-1859, TVSu-1823, and TVSu-2046, among others. In addition, TVSu-1943, TVSu-1930, and TVSu-1979 were among those identified as being near the vector for moisture content, and the accessions associated with protein were identified as being TVSu-2048, TVSu-2021, and TVSu-1923 among others. There were other accessions in close vicinity to the fat vector, including TVSu-1905, TVSu-1952, and TVSu-1899, all of which were identified as such. In terms of tryptophan content, accessions TVSu-2031 and TVSu-1764 were among those found to be associated, whereas accessions TVSu-1918, TVSu-1892, and TVSu-2025 were among those found to relate to tannin content. Phytate is associated with the accession TVSu-1863, which is one of the accessions associated with it.

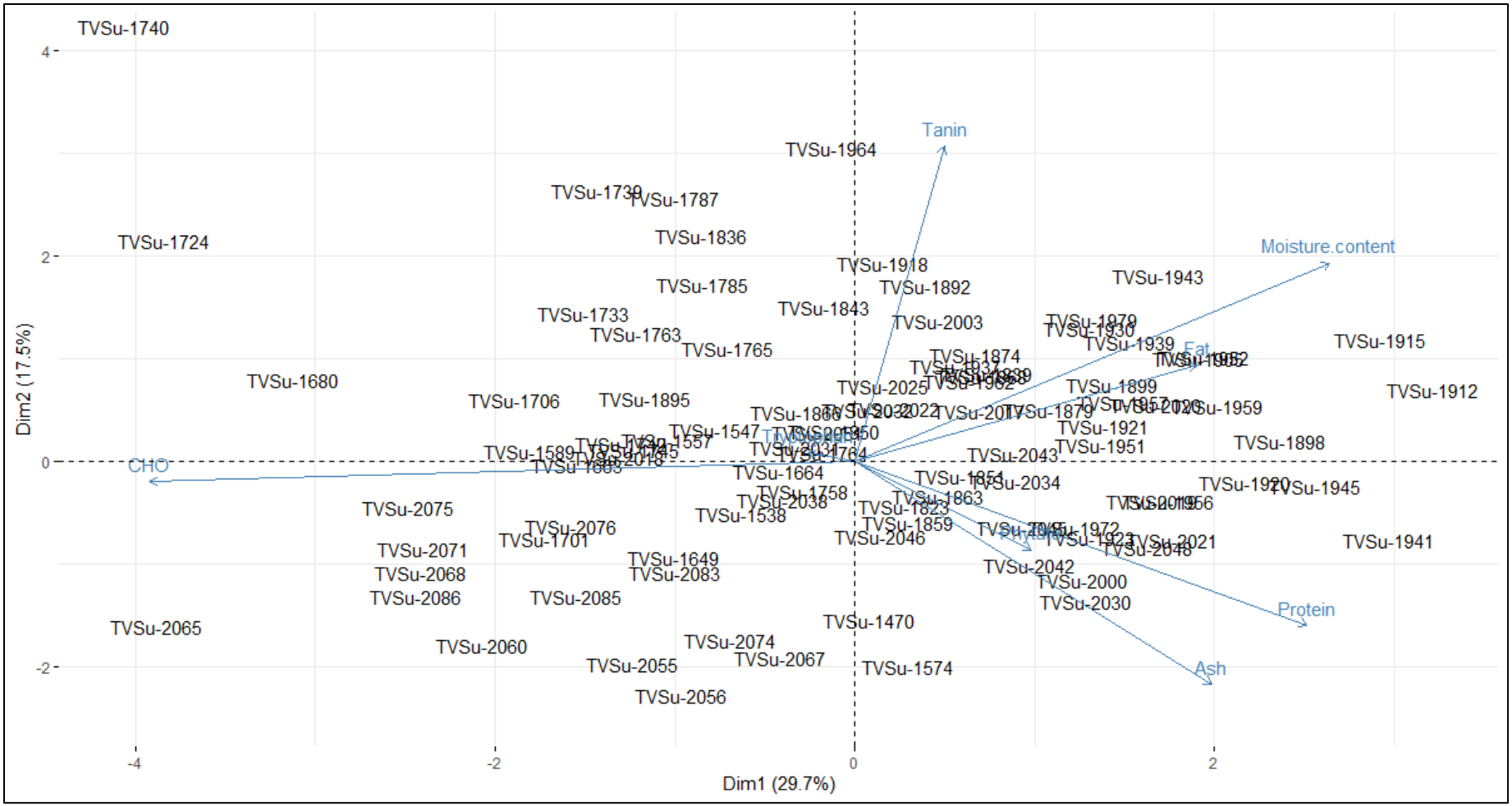

Fig. 5.1. Biplot showing the relationship between the different accessions with the nutrient and antinutrient compositions.

5.3.3 Clustering based on analyzed traits

The 95 accessions may be divided into four distinct clusters based on their nutritional and anti-nutritional features, according to hierarchical clustering (Fig. 5.2). Three clusters with similar numbers of accessions were identified. The first cluster had thirteen accessions, whereas the second cluster, with thirty accessions, was the largest. With twenty-five and twenty-seven accessions, the third and fourth clusters followed closely behind the second cluster. The first cluster was defined by accessions with high CHO mean values but low MC mean values. Accessions in the second cluster had high mean values for ash, fat, and phytate contents, whereas accessions in the third cluster had low mean values for phytate and fat but a high mean value for MC, and accessions in the fourth cluster had high mean values for tryptophan and tannin (Fig. 5.2).

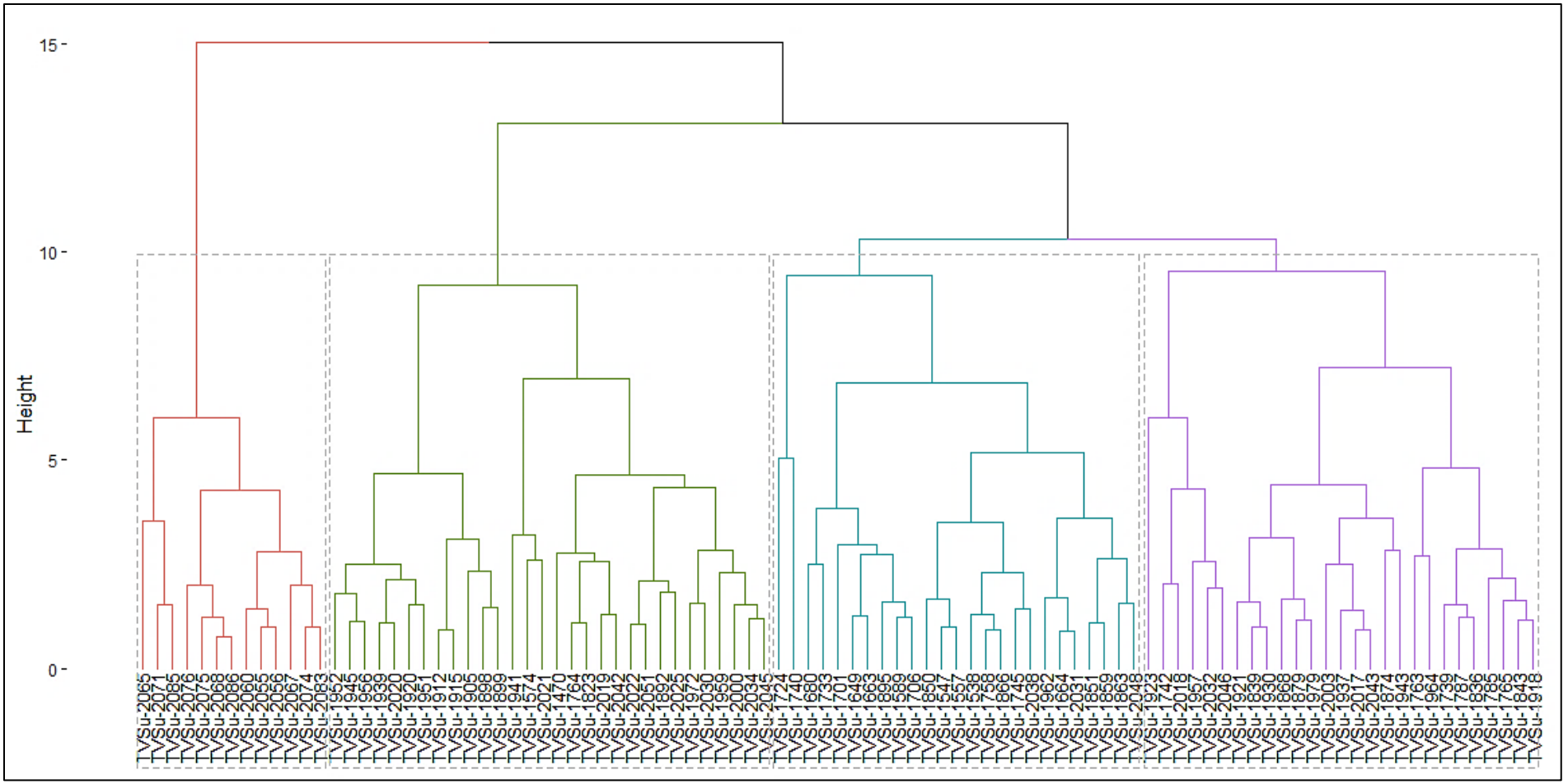

Fig. 5.2. Dendrogram categorizing the accessions according to their similarities for the nutrient and antinutrient traits.

5.3.4 Correlations among the nutritional and anti-nutritional components

In the bivariate correlations, it was discovered that the nutrients had varying degrees of association with one another (Fig. 5.3). Asterisks indicate that the correlations are statistically significant at a p-value of less than 0.05. Positive correlations are represented by the blue hues, whilst negative correlations are represented by the red colors. The deeper the hue, the greater the degree of negativity or positive represented by the matrix. Phytate was found to be strongly linked with protein ($r = 0.58$, $p<0.05$), fat ($r = 0.28$, $p<0.5$), and ash ($r = 0.58$, $p<0.05$) in the correlation matrix (Fig. 5.3). Tryptophan correlates negatively with protein content ($r = -0.17$, $p<0.05$), tannin ($r = -0.10$, $p<0.05$), and moisture content ($r = 0.43$, $p<0.5$). Fat correlates positively with moisture content ($r = 0.43$, $p<0.5$), and tannin ($r = 0.16$, $p<0.5$). The moisture content and CHO ($r = -0.83$) had the largest negative connection, followed by protein and CHO ($r = -0.77$), which had the second highest negative correlation. A positive correlation was found between phytate and protein ($r = 0.23$, $p<0.5$) as well as tryptophan ($r = 0.08$, $p<0.5$), while a negative correlation was found between phytate and tannin ($r = 0.21$, $p<0.5$). This means that the mount of phytate is not affected by the amount of tannin present.

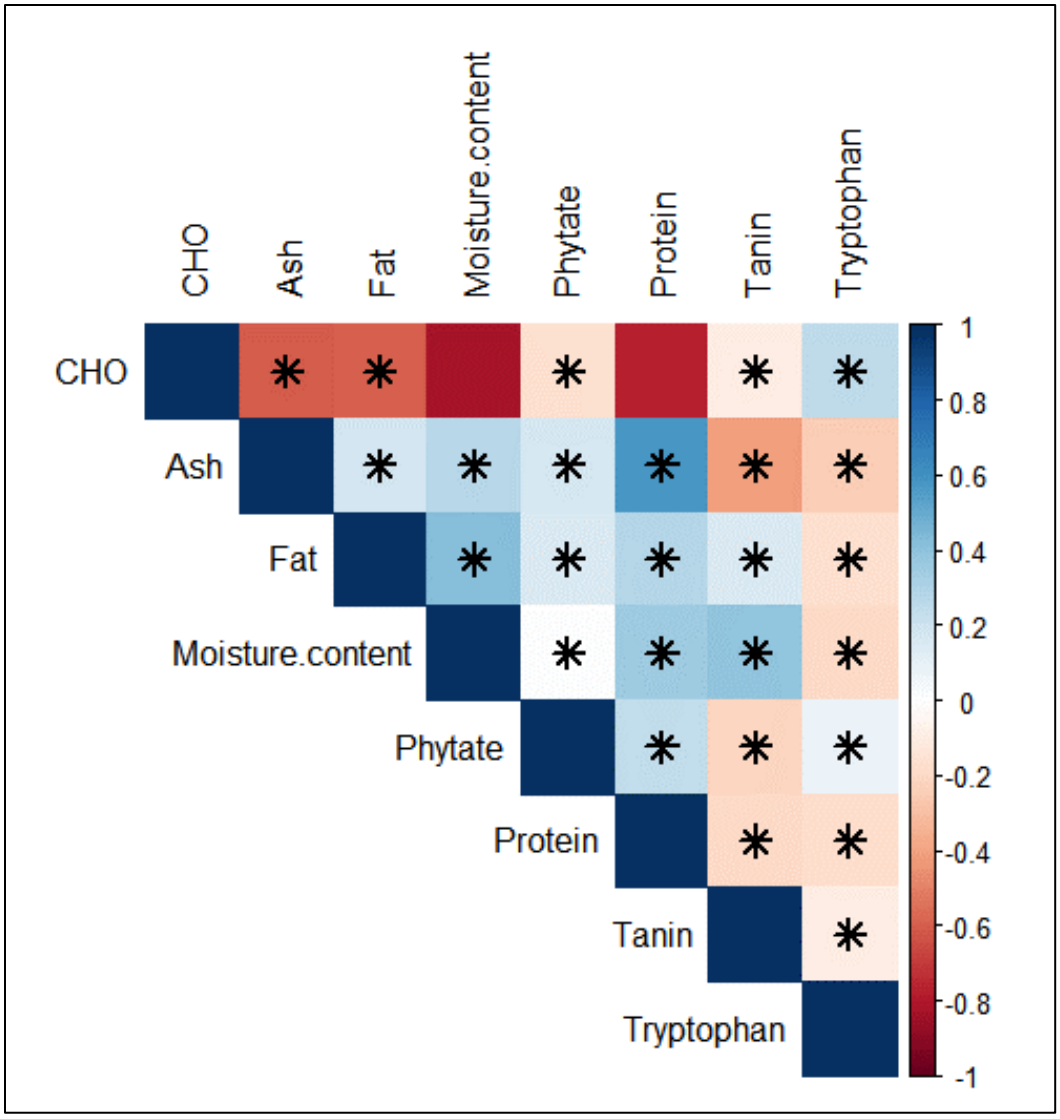

Fig. 5.3. Pearson's linear correlation coefficient of morphological traits studied

*= significant, P<0.05.

5.4 Discussion

Food insecurity in the developing world often results from low nutritional quality of some of the traditional food stuffs and high cost of protein foods high cost of fortified foods is not easily available for most families in the developing world (Ijarotimi and Keshinro, 2020) due to high level of poverty. Legumes have therefore come under immense importance in these regions as they

have been proven to be alternative sources to fortified foods for nutrients. Hence, the need to increase their level of consumption.

5.4.1 Genotypic variability in nutrient and antinutrient contents in BGN

Plant genetic, physiological, and morphological variations have a direct impact on breeding outcomes. Therefore, the importance of variations in different traits cannot be overemphasized. There are significant variances in nutrient and antinutrient contents amongst the accessions studied (Tables 5.2 and 5.3), therefore, they are excellent resources for developing or selecting parental lines for better nutritional performance.

Carbohydrates are needed for energy and are present in the form of starch and sugars varying from simple sugars to complex sugars i.e., the monosaccharides, disaccharides, and polysaccharides. According to Liu et al. (2020), pulse seeds are high in carbohydrate content. Therefore, it is expected that BGN have high amount of carbohydrate. In this study, the carbohydrate content of 58.34% is similar to the result reported in the study of Atoyebi et al. (2017) (range of 42.77% - 62.76%) and higher than the 50.2% reported by Yao et al. (2015). The higher the moisture content, the higher the susceptibility of the seeds to attack from microorganisms (Ijarotimi and Keshinro, 2020). Therefore, those seeds having low moisture content will have longer shelf life than those with high moisture content. Pulse seeds contain 20-40% protein (Bessada et al., 2019; Pandey et al., 2016), a value twice as much as that present in cereals. The value for protein content in this study which is below 25% is similar to that obtained by Nti (2009) who reported a range of 19.3% to 27.1% and Sirivongpaisal (2008) who reported 15.48% protein content. When compared with soybean, BGN is lower in protein content as seen reported in the study of Wijewardana et al. (2019) on soybean seeds under well-watered conditions. However, these values fall in the range obtained for protein contents in other pulses such as pea, chickpea, beans, lupin, and cowpea

(Barać et al., 2015; Bessada et al., 2019). Within the *Vigna* genus, BGN mean concentrations of the nutrient components are similar to those reported in cowpea and mungbean (Boukar et al., 2016; Halimi et al., 2019; Muñoz-Amatriaín et al., 2017). Tryptophan is a precursor of auxin, which is an essential growth hormone in plants (Olanrewaju, 2016; Olanrewaju and Babalola, 2019; Olanrewaju et al., 2017), and bioactive compounds such as nicotinamide, melatonin, kynurenine in humans (Friedman, 2018; Palego et al., 2016). The tryptophan level recorded in this study shows that BGN can help get the required amount of tryptophan in the quantity recommended by WHO which is 1.70, 0.85, and 0.66 for infants, children, and adolescents respectively (FAO, 2011). The tannin content reported in this study (0.09% - 0.41%) is lower than the 0.25% -2.27% reported by Ofori et al. (2001). However, the mean value of 0.20 is greater than 0.046 reported by Mazahib et al. (2013) in Sudan and 0.039 reported by Ijarotimi and Esho (2009) in Nigeria. It is however, lower than tannin content in faba beans which is between 8-9% (Akkad et al., 2019). Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase, and lipase, as well as to lower protein quality and interfere with iron absorption (Gemede and Ratta, 2014). Tanning can be detrimental to microbial enzyme activities, including cellulose digestion and intestinal digestion, if the tannin concentration in the diet becomes too high (Aletor, 1999). The utilization efficiency and ease of digestion of nutrients is affected by tannin contents present (Rahman et al., 2019). The strong anti-nutritional compound phytate is present in nearly all legumes and seeds (Gemede and Ratta, 2014). Phytic acid inhibits dietary tyrosinase, trypsin, pepsins, and lipases (Hendricks, 1989). The average phytic acid level reported by Mazahib et al. (2013) in Egyptian landraces was 14.78 mg/100g which is higher than the report in this study. The mean phytic acid content reported in this study is higher than that reported in the study of Yao et al. (2015) who reported a mean value of 1.1 mg/100g. Phytic acid

binds to ion complexes resulting to decrease in the concentration of these ions (Grases et al., 2017). Most of the phosphorus in phytic acid is unavailable to monogastric animals because they do not have the enzyme phytase (Akande et al., 2010). Furthermore, dehulling in some pulses have been shown to increase their phytic acid content but decreases tannin content because tannins are mainly found in the seed coats (Patterson et al., 2017).

5.4.2 Association among accession, nutrient, and anti-nutrient components

In addition, the PCA biplot showed four clear separations in the biplot according to the nutrient components (Fig. 5.1). Accessions high in carbohydrate are also high in tryptophan, while those high in tannin content are also high in moisture and fat contents but they are low in carbohydrate, tryptophan, protein, ash, and phytate contents. Likewise, those high in protein content are also high in ash and phytate contents but low in the other components. Considering the antinutrients, those high in phytates are low in tannin and *vice versa*. Tryptophans are amino acids constituting total proteins. They are therefore part of the total protein hence, accessions high in tryptophan are expected to be high in protein and correlate positively with total protein. In this study, however, accessions high in tryptophan are low in total protein and *vice versa*.

The hierarchical clustering (Fig. 5.2) agreed with the biplot result (Fig. 5.1), which showed that accessions with comparable nutritional and antinutrient contents were clustered together. Divergence among BGN accessions has been successfully identified using clustering based on nutritional and antinutrient components (Aremu et al., 2006; Hlanga et al., 2021; Ijarotimi and Esho, 2009; Mahala and Mohammed, 2010; Mazahib et al., 2013; Ofori et al., 2001; Onimawo et al., 1998; Yao et al., 2015). To avoid inbreeding depression, the selection of accessions for crossing should focus on divergent accessions, while proposing a number of accessions for production

might distribute the risk posed by biotic and abiotic pressures, particularly in stress-prone areas such as Sub-Saharan Africa.

5.4.3 Association between nutrient and anti-nutrient components

Finally, correlation among the nutrient and antinutrient components showed that breeding for nutritional components in these accessions will require careful selection processes. Although there are a large number of significant correlations, numbers of positive correlations are however like those of negative correlations. The negative relationship between carbohydrate and protein aligns with the result reported in the study of Ndidi et al. (2014) however, the opposite was reported for the relationship between ash and phytate.

The environment interacts with different plants to give different responses which is observed in nutrient, antinutrient components, and other traits such as reported in the studies of Fauziah et al. (2020); Ribeiro and Kläsener (2020); Vogelsang-O'Dwyer et al. (2020) because these traits are regulated by specific genes. The impact of the environment on these genes to affect the nutrient components was not taking into consideration in this study. Therefore, nutrient and antinutrient components from plants grown in various environments should be studied to ascertain the extent of the environmental influence on the nutrient and anti-nutrient components. Secondly, the emergence of NGS has revolutionized molecular studies in various areas, therefore, gene locus regulating these traits can be identified using technologies such as GWAS for improvement breeding programs.

5.5 Conclusion

In this study, two of the traits studied, *viz* carbohydrate and tryptophan are high in more than half of the accessions while the others have high amount of the remaining traits including the

antinutrient factors. Therefore, selection of accessions having low or minimal amount of anti-nutrient components can be made possible for future improvement. Variation in nutrient and anti-nutrient components in BGN observed here agrees with previous findings. The results of this study offer opportunities for selection and breeding of the crop for beneficial nutrient compositions and at the same time those with low amounts of antinutrient factors. Therefore, BGN can successfully improve nutritional security even more than some major crops.

5.6 CRediT authorship contribution statement

Oluwaseyi Samuel Olanrewaju: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Olaniyi Oyatomi:** Conceptualization, Investigation, Writing - review & editing, Supervision. **Olubukola Oluranti Babalola:** Conceptualization, Writing - review & editing, Supervision. **Michael Abberton:** Conceptualization, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

5.7 Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 6: ARTICLE 5

Molecular dissection of early vegetative stage Bambara groundnut drought tolerance using a genome-wide association analysis method

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Abstract

Water stress response in plants involves a repertoire of various reactions involving physiological, biochemical, and molecular responses. Hence, elaborating the complexity of the trait. Most crops are severely hampered by water stress. The high protein, nitrogen-fixing Bambara groundnut (BGN) is important in sustainable agriculture. Genetic resource collections are vital for breeding genetic diversity. The wooden box method is the most basic way to study large population water stress. The responses to water stress of 95 BGN accessions were examined using genome-wide association technology. A link was found between molecular markers and phenotypes. We used a two-way ANOVA with interaction between the accessions and the duration of stress exposure to better understand the responses. Even though both the duration and the accessions produced significant results individually, the ANOVA analysis found no significant interaction between them. As the stress increases, the accessions' responses differ significantly. The correlation analysis shows a positive correlation between chlorophyll content and recovery as stress progresses. To better understand the relationship between traits and molecular markers, we used the generalized linear model (GLM) and the mixed linear model (MLM) to analyze phenotypic and genotypic data using the Mungbean reference genome. Twenty significant SNPs (15 for GLM and 5 for MLM with the threshold for the association set at P of 1×10^{-3}) were associated with the studied morphological traits. Previously reported candidate genes for drought tolerance were identified. These are transaldolase, proline transporter 1, GDSL esterase, and salicylic acid-binding protein 2 genes. Candidate accessions for drought tolerance for BGN breeding can be selected from these accessions. Furthermore, promising validated markers can be used for marker-assisted selection to genetically improve drought tolerance through a breeding program.

Keywords: Bambara groundnut; candidate genes; GWAS; marker-trait association; water stress responses

6.1 Introduction

The increasing population, urbanization, and the decrease in agricultural land pose a great threat to food and nutrition security. Lack of access to food is expected to increase by 2050 due to the projected increase of the human population to exceed 9 billion [1]. All these pose a great threat to human development and there is a need for immediate solutions through innovative approaches to increase food production.

Water is a very important component for the existence of all life. All living things need water for survival in all stages of their development. Plants need water for the transport of mineral elements and nutrients, they also need water for vigor and other important processes. Unavailability of water either in little or large quantity affects the physiological and biological processes in plants. Most plants have little or no chance of survival in water-deficient conditions. Under drought stress, systematic responses are induced in plants that interact with plant growth, development, physiology and biochemistry, active oxygen metabolism, signal transduction, and regulation of gene expression [2,3]. Climate change impact is also adding to the reduction in available water for plant use. Changes in temperature, humidity, and rainfall pattern all affect the amount of water available, thus having a major say in crop production and output. From seedling to mature plants, water is essential throughout the lifecycle of plants. Coupled with innovative strategies, promotion, and improvement of plants that can naturally withstand water-limiting stress can be adopted to combat the challenges posed.

In the vegetative stage, plants they require water for production because this is the beginning of the reproduction period in the plants. Whatever happens to them at this stage will affect their yield, therefore, this is a very critical stage in the development of plants. Flowering starts at this period, and flowering starts the period of pollination, fertilization, and subsequently seed formation. Without the flowers, there cannot be any yield from the plants.

BGN is one of such plant that is reported to have the ability to tolerate or resist drought [4-6]. They make use of one of the three mechanisms of drought response in their attempt to survive during periods of water scarcity. The vegetative period in this plant usually begins around 4 weeks after planting. It is majorly cultivated by subsistence farmers in the developing world especially sub-Saharan Africa and some parts of Asia [7]. The plant is high in protein and other nutrients when compared to the major food crops. As a result, it will complement well in place of the major food crop counterparts when fully integrated into the food system. The ability of this plant to withstand water stress makes it an ideal plant to help in achieving food and nutritional security in the arid and semi-arid regions.

The challenges with breeding and research of most underutilized crops such as BGN have limited the development of new varieties. Due to the limitation in developing new varieties, Bambara groundnuts are still mostly cultivated as landraces [5,8-12]. The development of high-yielding and environmentally-stable varieties of the crop will help in sustainably achieving food and nutrition security. Identification of important traits of interest in crops for improved molecular breeding has been accelerated with the advent of Next Generation Sequencing (NGS) technology. Molecular markers have been developed for marker-assisted selections of good varieties of crops, genome-wide association studies (GWAS) have also been employed on many major food crops for molecular mechanisms of traits from genotype to phenotype [13-17]. Location of genes (through

quantitative trait loci (QTL) mapping and genome-wide association study (GWAS) analysis) responsible for traits of interest has been employed in various studies [18-20], and this has helped in improving the productivity of various major crops and development of some lesser grown crops. The result of this has frequently been the production of new and improved varieties toward important traits such as disease resistance, drought tolerance, and improved yield, among others, as reported in the studies of Jiang, et al. [21,]Kumar, et al. [22,]Khattak, et al. [23], and Sheoran, et al. [15]. The use of molecular markers has greatly improved plant breeding. Various markers have been used in various crops for selection and improvement. One of such molecular markers is the diversity arrays technology (DArT) markers which have been used in various crops of importance [24-26].

Application of GWAS to BGN for drought tolerance trait is a major step in the improvement of this crop. Small nucleotide polymorphisms (SNPs) will be generated to identify the regions of the genome that control the response of the crop to water stress. The main physiological responses of plants to water stress are leaf wilting, senescence, and change in stem color. Chlorophyll content is another parameter that is affected by water stress. The chlorophyll content and the amount of available water are closely related [27]. The lack of water in leaves influences the production of chlorophyll. This causes decomposition of chlorophyll which is evident by the yellowing of leaves.

Food security can be achieved in different ways but due to the unavailability of a well-annotated BGN reference genome, this study focuses on identifying the region of the genome responsible for drought response through GWAS for the possible improvement of the crop using the well-annotated Mungbean genome [28] as the reference genome. The aims of this study are to characterize the responses to drought stress in these accessions, to identify the regions of the genomes

controlling for drought stress, and finally the candidate genes controlling for the drought stress traits using GWAS.

6.2 Results

6.2.1 Summary statistics and ANOVA analysis

Moisture content in the boxes showed its minimum at the period of stress and increased again after stress period. The summary statistics likewise presented in Table 6.1, showed the mean, variance, standard deviation, minimum and maximum values of each trait scored throughout the experiment. Chlorophyll content had maximum and minimum values of 99.2 and 5.9 which were recorded on the 7^{th} and 13^{th} days after the onset of stress respectively while wilting and stem greenness had 5 and 0 respectively (Fig. 6.1). Leaf senescence had a maximum and minimum score of 2 and 0 while recovery was between 0 and 1 (Fig. 6.1). The ANOVA table (Table 6.2) presented the influence of the accessions and the effect of the durations of stress on the trait responses. In addition, the interaction between the accessions and the duration of stress i.e., accession-stress duration interaction, on the response traits was also presented. For all traits, the significant effects of the accessions were substantial while the days was also significant for all traits except stem greenness. Furthermore, we decided to examine the significance of the interaction between the accessions and the days on the traits and result showed that they were not significantly substantial. Diverse levels of statistically significant responses are seen. Chlorophyll content is seen to reduce as the days of stress increased. Water is essential for various transportations in the cell therefore, low level of water affects the amount of chlorophyll present in the leaves. Like the chlorophyll content, recovery rate also decreased as the duration of stress increased. Therefore, recovery rate is positively correlated with the chlorophyll content. The rate of wilting, leaf senescence, and stem greenness on the other hand, increased with the duration of stress.

Table 6.1 Summary statistics of the traits scored across all environments.

Traits	Minimum	Maximum	Mean	Variance	Standard deviation
CHLCON	5.90	99.20	35.66	151.38	12.30
WTN	0.00	5.00	2.97	1.68	1.30
LS	0.00	2.00	0.90	0.61	0.78
SG	0.00	5.00	2.28	5.26	2.29
Rec	0.00	1.00	0.29	0.21	0.46

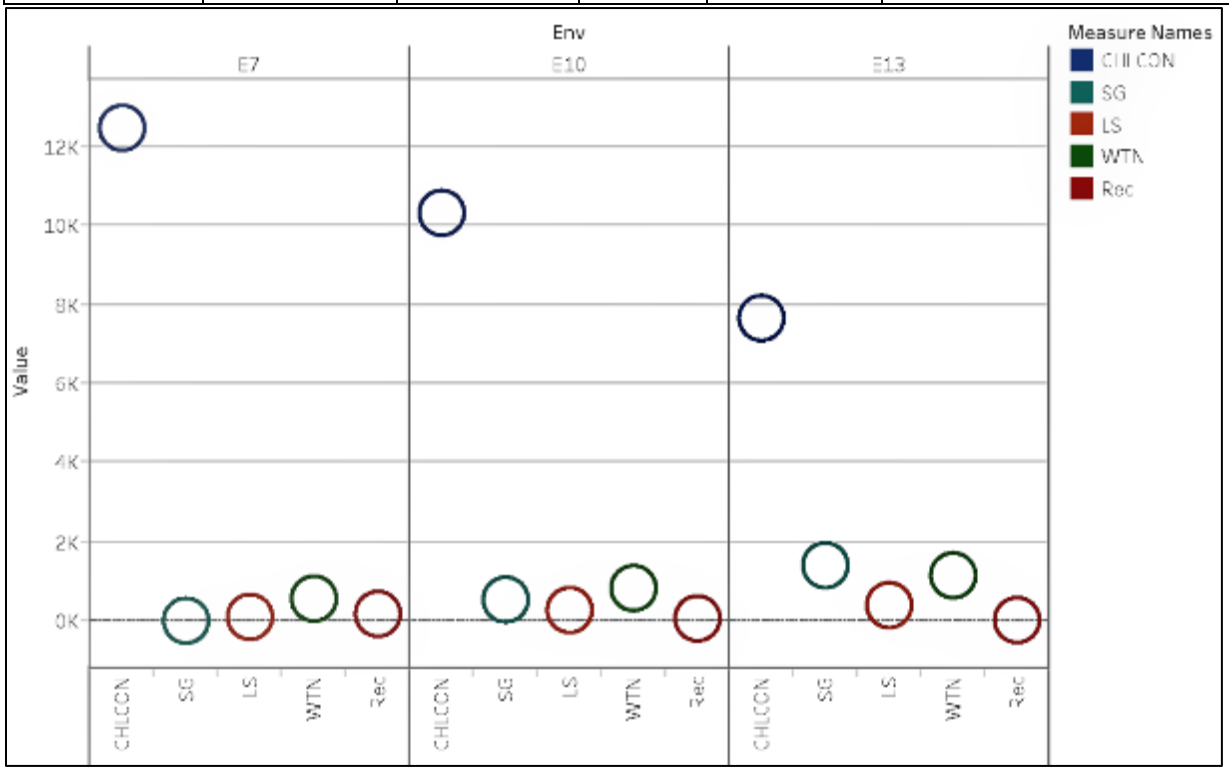

Fig. 6.1. Traits' summary statistics for each environment. An environment represents days after stress imposition.

Table 6.2 Overall ANOVA for the performance of the accessions at different days of water stress and their interaction responses.

Source of variations	DF	CHLCON	WTN	LS	SG	Rec
Accns	94	125.9·	1.929***	0.703***	1.18***	0.1984·
Day	2	20433.6***	305.267***	77.699***	1756.83^{ns}	23.0959***
Accns*Day	188	107.1^{ns}	0.433^{ns}	0.289^{ns}	1.08^{ns}	0.1231^{ns}
Residuals	570	99	0.989	0.434	1.17	0.1567
GRANDMEAN		35.66	2.97	0.89	2.27	0.29
LSD		9.21	0.92	No result	1	0.37
CV		0.345	0.437	0.875	1.008	1.552

†Accns=accessions; LSD=least significant difference; CV=coefficient of variation; CHLCON=chlorophyll content; WTN=wilting; LS=leaf senescence; SG=stem greenness; Rec=recovery
***Highly significant at $p < 0.0001$
· Significant at $p < 0.05$
ns = no significance

6.2.2 Principal component analysis

Looking at the variation in the stress responses among the 95 BGN accessions, the first two principal components (PC) with Eigen values ≥1 described around 77.7% of the total variations observed (Table 6.3). PC1 (Dim.1) and PC2 (Dim.2) accounted for 52% and 25.737% of the variations respectively in the data set under consideration (Fig. 6.2).

Table 6.3 Contributions of traits to the principal components' axis.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
CHLCON	4.13	53.97	41.41	0.09	0.40
WTN	26.93	4.38	0.96	65.66	2.07
LS	27.73	6.19	3.14	8.52	54.43
SG	28.27	4.32	0.03	25.68	41.71
Rec	12.95	31.14	54.46	0.06	1.39
Eigenvalue	2.598	1.287	0.475	0.356	0.284
Variance (%)	51.968	25.737	9.500	7.112	5.683
Cumulative variance (%)	51.968	77.705	87.205	94.317	100.000

The various links between accessions and traits are depicted in Fig. 6.2. Those accessions whose vectors were plotted close to the vector for a specific trait were highly correlated to that trait, while the length of a vector for an accession will estimate its mean for that specific trait. An accession that was drawn along with a lengthy vector implies that it had a high mean value for the trait that it related to. Therefore, SG (28.27%), LS (27.73%), and WTN (26.93%) were the highest

contributors to PC1 while CHLCON (53.97%) and Rec (31.14%) were the highest contributors to PC2 (Table 6.3). For traits WTN, SG, and LS, the top ten accessions that were strongly connected include TVSu-2067, TVSu-1538, TVSu-1758, TVSu-2055, 2017, TVSu-1843, TVSu-1745, TVSu-1866, TVSu-1952, and TVSu-1972, while those strongly connected to CHLCON and Rec were TVSu-2019, TVSu-2076, TVSu-1915, TVSu-2025, TVSu-1979, TVSu-2003, TVSu-2071, TVSu-2048, 1868, and TVSu-2064.

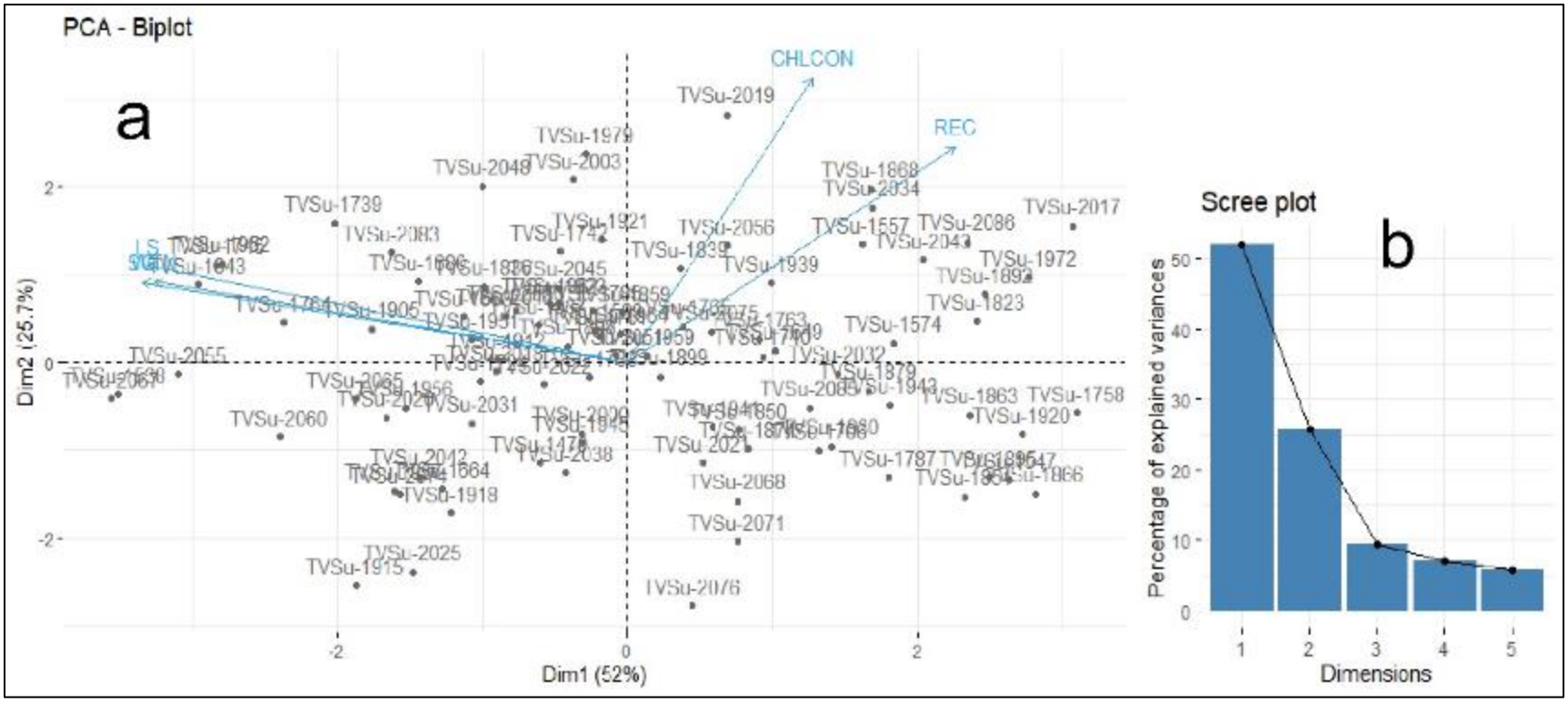

Fig. 6.2. Biplot and scree plot visualization. (a). Biplot at indicated by different colors for the days after stress was induced. (b). Scree plot showing the contributions of the dimensions to the biplot.

6.2.3 Clustering of accessions based on phenotypic trait responses

The 95 accessions may be divided into four distinct clusters based on their stress responses according to the hierarchical clustering (Fig. 6.3a). The colors imply negative correlations (red) and positive correlations (blue) (Fig. 6b). The blue cluster had 47 accessions, followed by the purple cluster with 21 accessions, the green cluster with 17 accessions, and finally the red cluster with 10 accessions. Accessions in the blue and purple clusters were majorly characterized by high level of stem greenness, wilting, and leaf senescence. Hence, these accessions can be said to be more susceptible to drought stress impact. However, accessions in the red and green clusters had high level of recovery and high chlorophyll content. Selecting accessions in these clusters can improve BGN breeding for drought tolerance and recovery. The cluster analysis was also done for the accessions for each day i.e 7th, 10th, and 13th day that data were taken.

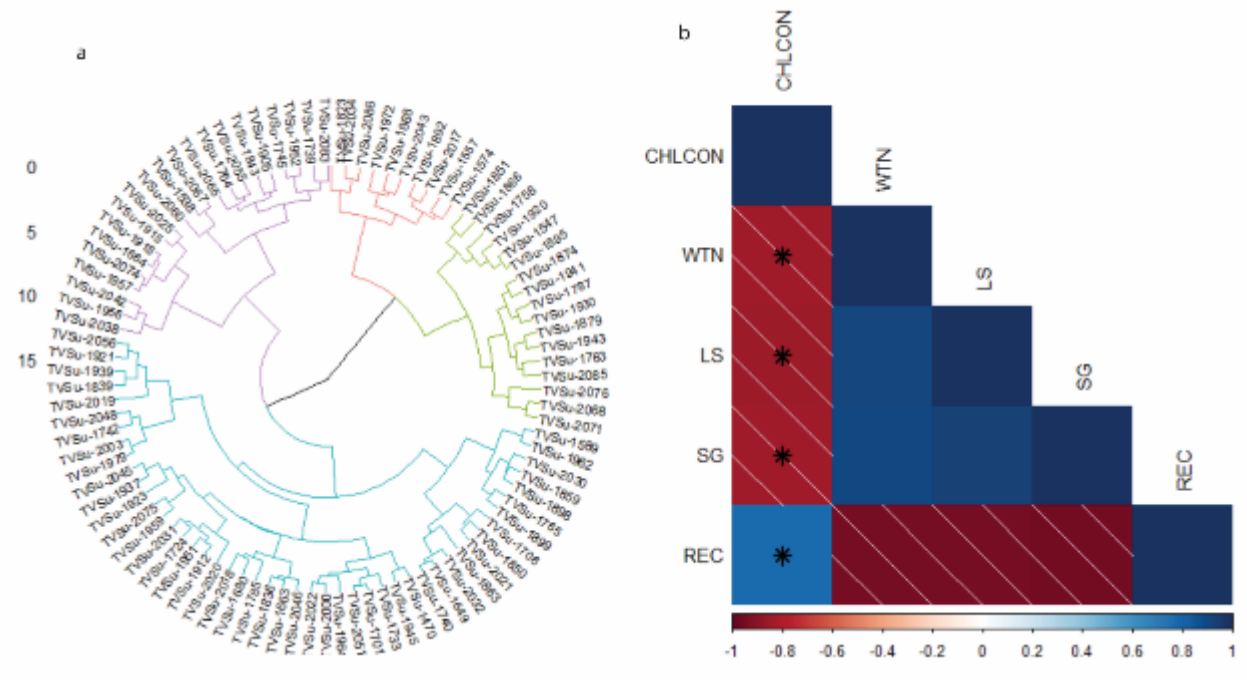

Fig. 6.3. Clustering and correlation analysis (a) Clustering using Ward.D method to group the accessions based on the stress response traits (b) Correlation matrix showing the relationship among the variables throughout the duration of water stress.

In the bivariate correlations for the morphological stress-response traits, it was discovered that the nutrients had varying degrees of association with one another for the combined days (Fig. 6.3b) and individual days data was taken (Appendix). Asterisks indicate that the correlations were statistically significant at a $p \leq 0.05$. Positive correlations were represented by the blue color, whilst negative correlations were represented by the red color. The deeper the color, the greater the degree of negativity or positive as represented by the matrix. CHLCON correlated positively and significantly with only REC ($r = 0.78$, $p \leq 0.5$) and negatively with WTN ($r = -0.85$, $p \leq 0.5$), LS ($r = -0.85$, $p \leq 0.5$), and SG ($r = -0.85$, $p \leq 0.5$). WTN correlated positively but not significantly with LS ($r = 0.91$) and SG ($r = 0.90$), but correlated negatively with REC ($r = -0.95$). Finally, LS and SG also had a positive correlation ($r = 0.93$).

6.2.5 Phylogenetics, population structure, and genome wide association analysis

The first three components accounted for about 41.2% of the total diversity with PC1 having 24.9%, PC2 with 10.4%, and PC3 with about 0.06% (Fig. 6.4a). These three PCs were significant in the population structure of the accessions. The scree plot showed the percentages explained by each PC (Fig. 6.4b). Neighbor-joining analysis grouped the accessions according to their region of origin (Fig. 6.5a) and the pairwise kinship matrix was represented in a heatmap (Fig. 6.5b).

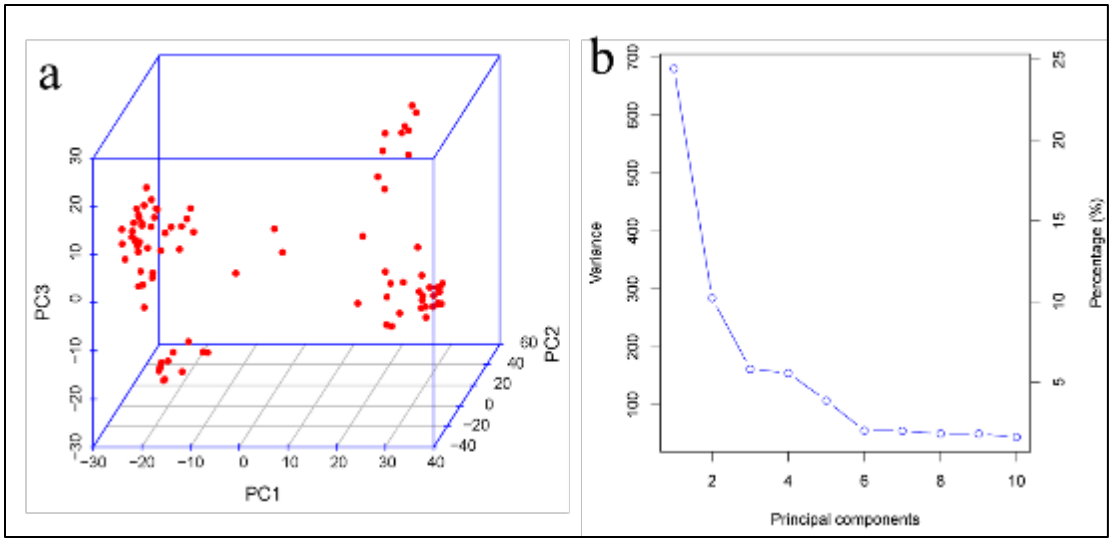

Fig. 6.4. PCA and scree plot showing the distribution of the components.

(a). three-dimensional plot of the distribution of the accessions along the first three principal components (b). scree plot depicting the number of significant PCs.

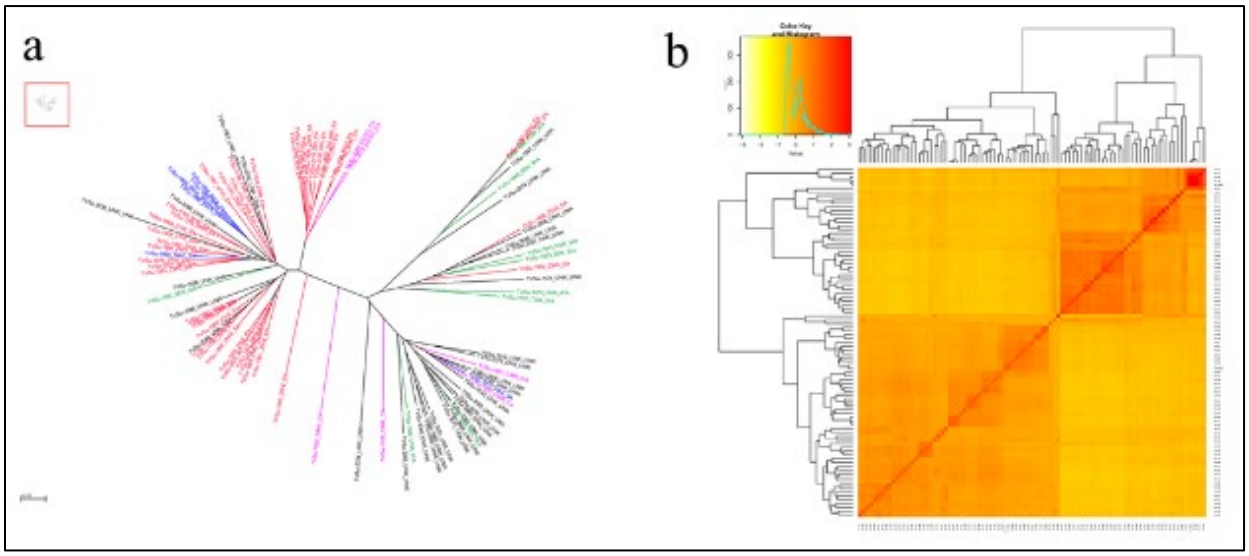

Fig. 6.5 Clustering and heatmap (a). clustering of the accessions based on the SNP panels using the neighbor-joining method. (b). heatmap of pairwise kinship matrix of the 93 accessions.

A total of twenty significant SNPs (considering thresholds of log (p) ≤ 0.001 with $R^2 \geq 9$ %) from both GLM (15) and MLM (5) models were identified by GWAS analysis of the BGN accessions in response to water stress using the Mungbean reference genome [28] (Fig. 6.6, Table 6.4). Both models identified same QTLs on chromosome 3 for wilting, chromosomes 2 and 4 for stem greenness, and chromosome 10 for leaf senescence (Fig. 6.6a). Chromosome 2 have two QTLs for

leaf senescence and stem greenness, chromosome 3 have two QTLs for leaf senescence, two QTLs for wilting, and one QTL for stem greenness, chromosome 4 have QTLs for chlorophyll content, stem greenness, and wilting, while chromosome 7 has QTLs for chlorophyll content and stem greenness. Finally, two QTLs were detected for leaf senescence on chromosome 10 (Fig. 6.6a). The GLM model, identified 6 positions for chlorophyll content, 5 for leaf senescence, 4 for stem greenness, and 1 for wilting, while the MLM model did not identify any significant SNP at the threshold value for chlorophyll content, however, 1 position was identified for leaf senescence, 2 for stem greenness, and 2 for wilting (Table 6.4). Both models did not identify any significant association for the trait "recovery" at the threshold level. The most significant association [–log10(p) = 0.000310879], with the highest allelic effect of +2.42, was detected for chlorophyll content and was associated with the marker 24384749|F|0-12:G>C-12:G>C located on chromosome 5 (Table 6.4). GWAS analysis revealed QTLs that affected traits positively under drought. Overall, the result suggest that drought-responding QTLs associated with chlorophyll content were located on chromosome 4, 5, 6, and 7, while QTLs associated with leaf senescence were located on chromosomes 2, 3, and 10. Drought responding QTLs associated with stem greenness were located on chromosomes 2, 3, 4, and 7 and finally, those associated with wilting were located on chromosomes 3 and 4. This information might be useful for selection of drought tolerant accessions and help to improve the understanding of genetics mechanisms of plants exposed to drought stress.

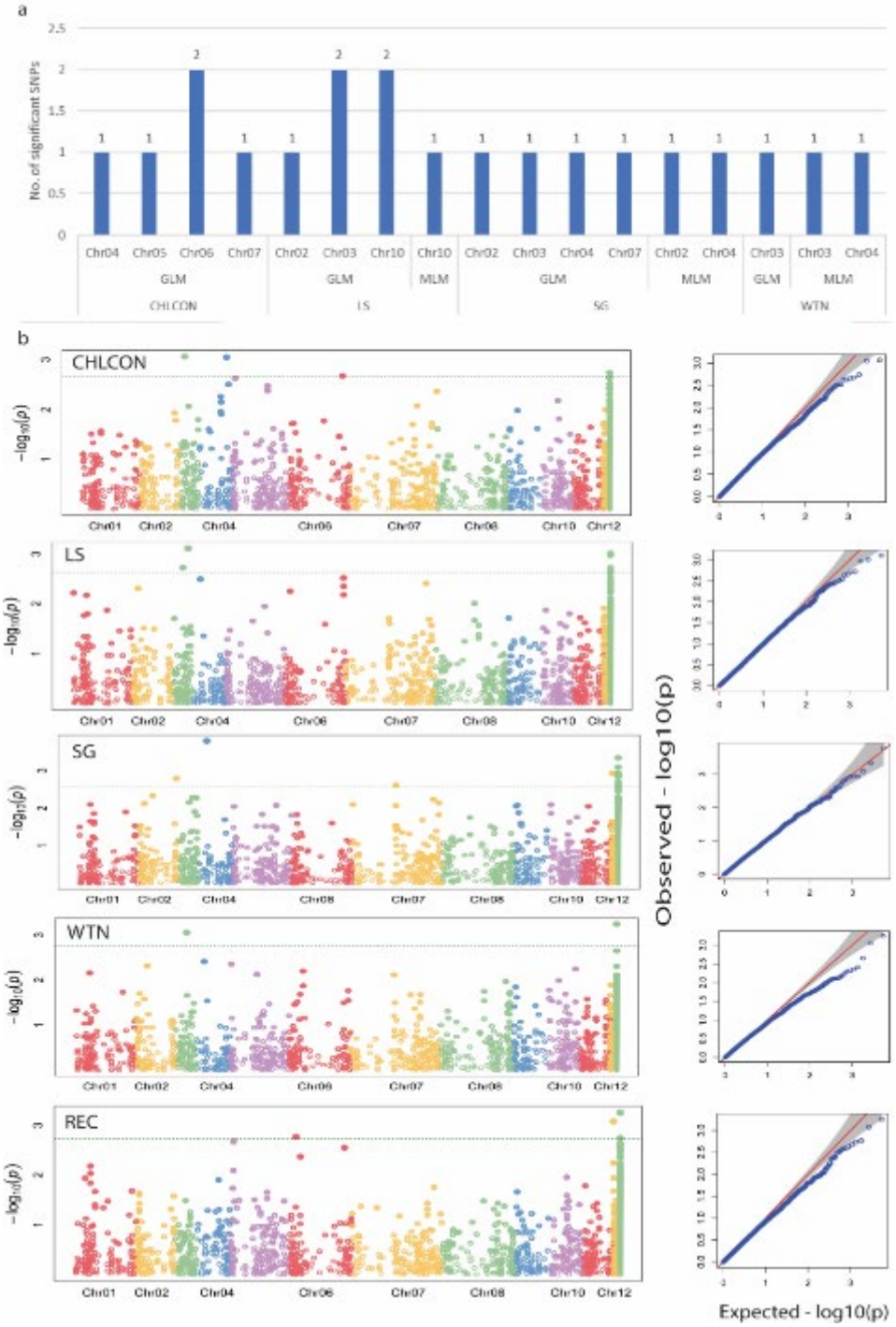

Fig. 6.6 SNP distributions and Manhattan plots (a) Significant SNP distribution on each chromosome by both models for each trait at p <0.001. (b) Manhattan plots and Q-Q plots for the traits observed in the study.

Table 6.4 SNPs associated with water stress response traits at a significant level of 0.001.

GLM						
Traits	**SNP markers**	**Chr**	**Position**	***P-value***	**R^2**	**Allele effects**
CHLCON	27641298|F|0-47:A>G-47:A>G	Chr07	54956049	0.000176331	17.0358663	-2.163264691
CHLCON	24384749|F|0-12:G>C-12:G>C	Chr05	1843979	0.000310879	15.6504868	2.418827751
CHLCON	24385086|F|0-68:A>G-68:A>G	Chr06	5711734	0.000393729	15.0799968	-1.500327511
CHLCON	27640350|F|0-31:C>T-31:C>T	Chr06	3535765	0.000467425	14.6682111	-2.125349099
CHLCON	24385377|F|0-39:G>A-39:G>A	Chr04	17021666	0.000960713	12.9625849	-3.47528109
LS	4183017|F|0-63:T>C-63:T>C	Chr03	6850679	0.000338981	20.5692084	0.133014646
LS	4181526|F|0-18:T>G-18:T>G	Chr10	18576117	0.000868651	18.4310677	-0.122404286
LS	4181674|F|0-38:T>G-38:T>G	Chr03	10147417	0.001000995	18.1142774	-0.163525936
LS	37312362|F|0-59:G>T-59:G>T	Chr10	3909869	0.001292491	17.5470139	0.324236991
LS	4175830|F|0-22:G>A-22:G>A	Chr02	6900474	0.001341007	17.4656185	-0.136437018
SG	4176293|F|0-46:G>A-46:G>A	Chr04	4735913	0.000197572	16.7561619	0.47545679
SG	24384561|F|0-54:G>C-54:G>C	Chr02	23113339	0.000486815	14.5709719	-0.408970588
SG	24386055|F|0-27:C>A-27:C>A	Chr07	26915780	0.000756935	13.5227548	-0.360987654
SG	4183017|F|0-63:T>C-63:T>C	Chr03	6850679	0.001418793	12.0556813	0.150066667
WTN	4183017|F|0-63:T>C-63:T>C	Chr03	6850679	8.59E-05	18.8251623	0.239088889
MLM						
Traits	SNP markers	Chr	Position	*P-value*	R^2	Allele effects
LS	4181526|F|0-18:T>G-18:T>G	Chr10	18576117	0.001212554	19.0373321	-0.128621833
SG	4176293|F|0-46:G>A-46:G>A	Chr04	4735913	0.000163179	18.4831925	0.481851716
SG	24384561|F|0-54:G>C-54:G>C	Chr02	23113339	0.001283485	13.6188491	-0.39707643
WTN	4183017|F|0-63:T>C-63:T>C	Chr03	6850679	0.000139751	18.998633	0.276916175
WTN	4176293|F|0-46:G>A-46:G>A	Chr04	4735913	0.001422478	13.5315573	0.515567084

(Chr.) The chromosome on which the marker was detected

(Pos*) Position (genetic distance cM) of the marker on the Mungbean chromosome - [28]

R^2* Phenotypic variation explained by marker

(-) the allele decreases the trait, while (+) the allele increases the trait

6.2.6 Putative candidate genes

Remarkably, we found SNPs positioned within or near genomic regions coding for proteins involved in drought responses (Table 6.5). Insights into the details of drought stress response and information on the genes and enzymes involved in this process may lead to innovative strategies to breed drought tolerant crops. In the present study, we identified twelve SNPs associated with drought stress response. These SNPs were co-localized with the *Vradi07g31020*, *Vradi05g01630*, *Vradi06g04840*, *Vradi06g03310*, and *Vradi04g08510* genes which encode for a transaldolase, pectin esterase, proline transporter 1, GDSL esterase, and outer plastidial membrane protein porin respectively. As well as the *Vradi03g05310*, *Vradi10g10930*, *Vradi03g08520*, and *Vradi02g06260* genes encoding for cell division cycle 20.2- cofactor of APC complex, putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase, UDP-glycosyltransferase 76F1, and SHUGOSHIN 2 respectively. Finally, *Vradi04g01720*, *Vradi02g02440*, and *Vradi07g10090* genes which encodes origin of replication complex subunit 1A, alanine--tRNA ligase, and salicylic acid-binding protein 2 were identified (Table 6.5). Transaldolase, proline transporter 1, GDSL esterase, and salicylic acid binding protein 2 have been previously reported to be involved with drought stress tolerance in plants.

Furthermore, most candidate genes identified in this study were associated with chlorophyll content, followed by leaf senescence and stem greenness. Four candidate genes were identified on chromosome 3 indicating that higher number of drought response genes may be located on this chromosome. SNP *Vradi03g05310* is associated with candidate genes controlling three traits (leaf senescence, stem greenness, and wilting) and SNP *Vradi04g01720* is also associated with candidate gene controlling two traits (stem greenness and wilting). Association of leaf senescence, stem greenness, and wilting with same genes supports the result from the correlation analysis

where these traits are positively correlated with one another (Fig. 6.3b). Remarkably, the identified candidate genes have roles in reactive oxygen species homeostasis, amino acid transport, cell signaling, and during seedling development and are expressed in different plant tissues.

Table 6.5 Putative candidate genes and their annotations. Candidate genes were chosen when the entire sequence overlapped with a marker sequence of highly significant QTLs. Physical position on the chromosome, the interval of the genes and their annotation are given.

Associated traits	**Candidate genes**	**Chr**	**Start**	**End**	**Annotation**
CHLCON	*Vradi07g31020*	Chr07	54954600	54957370	transaldolase
CHLCON	*Vradi05g01630*	Chr05	1843629	1845706	probable pectinesterase/pectinesterase inhibitor 12
CHLCON	*Vradi06g04840*	Chr06	5709817	5712822	proline transporter 1
CHLCON	*Vradi06g03310*	Chr06	3534825	3536853	GDSL esterase/lipase At1g71250-like
CHLCON	*Vradi04g08510*	Chr04	17018081	17021774	outer plastidial membrane protein porin
LS	*Vradi03g05310*	Chr03	6850396	6852752	cell division cycle 20.2, cofactor of APC complex
LS	*Vradi10g10930*	Chr10	18575423	18580620	putative tRNA (cytidine(32)/guanosine(34)-2'-O)-methyltransferase
LS	*Vradi03g08520*	Chr03	10145934	10148074	UDP-glycosyltransferase 76F1
LS	*Vradi02g06260*	Chr02	6899516	6902742	SHUGOSHIN 2
SG	*Vradi04g01720*	Chr04	4735371	4741234	origin of replication complex subunit 1A
SG	*Vradi02g02440*	Chr02	2291956	2316595	alanine--tRNA ligase
SG	*Vradi07g10090*	Chr07	26914778	26916028	salicylic acid-binding protein 2-like
SG	*Vradi03g05310*	Chr03	6850396	6852752	cell division cycle 20.2, cofactor of APC complex
WTN	*Vradi03g05310*	Chr03	6850396	6852752	cell division cycle 20.2, cofactor of APC complex
WTN	*Vradi04g01720*	Chr04	4735371	4741234	origin of replication complex subunit 1A

Chr: chromosome; CHLCON: chlorophyll content; LS: leaf senescence; SG: stem greenness; WTN: wilting

6.3 Discussion

This study estimated the responses of BGN accessions to five drought response traits to understand underlying genetic mechanisms. To the best of our knowledge, it is the first publication to identify associated SNPs for drought tolerance in BGN via GWAS. Furthermore, it stands as the first GWAS for BGN irrespective of the trait. The findings are essential for developing markers for molecular-assisted breeding approaches for climate-change resilience breeding objectives in BGN.

Water stress is one of the major factors in low crop yield. Inability of crops to get enough water required for metabolism and nutrient transport lead to reduced growth and low yield if death did not occur. Therefore, it is imperative that in this era of climate change, issue of water stress on crop productivity should be taken seriously. Crops that thrive better in the presence of low amount of water should be promoted and developed as one of the solutions to this challenge. On this note, the results in this study shows variability in the responses of the selected accessions when exposed to varying degree of water stress. Excess biosynthesis of chlorophyll leads to production of reactive oxygen species and oxidative stress [29]. However, a decrease in chlorophyll content during water stress is due to the damage caused by reactive oxygen species on the chloroplast [30].

Consistent with results in previous studies, increase in water stress leads to increase in wilting, leaf senescence, and reduction in chlorophyll content and stem greenness [30-34]. A lot of metabolic reorganization is needed for plants to get back to their normal state of growth after water stress. Few of the accessions in this study recovered after the stress period. In agreement with other studies [35-37], different accessions/genotypes respond differently when exposed to stress, therefore the different genetic make-up of each accession accounts for the different responses observed. Although there were no significant interaction between the accessions and days of stress in the

ANOVA (Tables 6.3 and 6.4), however, each accession and days showed substantial significance affect. Similar findings were reported by Mwale, et al. [30]. This further buttress the variability in the responses of the accessions which can be accounted for by the different mechanisms employed by each accession. From the PCA and clustering of the physiological responses, a clear impact of the stress severity at each stage was evident as the accessions were clearly separated (Figs. 2 and 3a). This further suggest that different mechanisms existed between accessions [38,39]. In the correlation analysis, a significant positive correlation between chlorophyll content and recovery was observed (Fig. 3b). This result is supported by the study of Chen, et al. [40] in which they also clarified that maintaining a higher but not excessive amount of chlorophyll is needed by plants for recovery.

Understanding the genetic basis of water stress response is important in breeding for drought tolerance in BGN. The identification of SNPs associated with water stress response will help in understanding the genetic response of BGN to water stress and facilitate genetic improvement and identification of drought resistant accessions. GWAS is powerful for identifying genetic loci of trait variations in plants. Although it often requires high density markers and large populations coupled with good statistical model [41], it however performs well in low populations. Furthermore, GWAS performance relies on target trait region, plant species, and platform used for analysis [42]. GWAS have been successfully applied in small populations and useful results have been reported in many studies [42-44].

SNP markers are codominant, polymorphic, and more useful for intra-accession genetic variations in contrary to biochemical markers [45]. GWAS analysis revealed several genetic regions controlling traits of interest for drought stress response. In breeding, significant markers can be used for MAS to identify drought tolerant accessions, especially when they have been detected already in

previously studies. Twenty significant SNPs for water stress response for studied traits were identified in this study.

On chromosome 7, the putative candidate gene *Vradi07g31020* was identified and it encodes transaldolase gene. The expressed protein, a hydrolase, is a key enzyme in the pentose phosphate pathway and it is involved in the synthesizes of ribose 5-phosphate [46,47]. The whole process produces reductive elements such as nicotinamide adenine dinucleotide phosphates (NADPH) precursors for the synthesis of nucleotides. In plants, various isoenzymes exist that may catalyze the reaction in the cytoplasm or inside plastids. Moehs, et al. [48], reported the increased expression of the protein in response to wounding in potato. Rong, et al. [46] also reported that drought can induce the expression of transaldolase gene. According to Zheng, et al. [49], it helps in maintaining cell homeostasis by maintaining reactive oxygen species balance.

Moreover, a proline transporter 1 (PROT1) is encoded by the candidate gene *Vradi06g04840.* Stress-induced accumulation of proline lowers the water potential of the cell in a process known as osmotic adjustment, thereby promoting water retention in the plant without interfering with normal plant metabolism [50]. This maintains cell turgor pressure and increases plant's survival under drought stress. Proline transporters regulate the distribution of proline throughout the plants. Up-regulation of proline transporter genes under drought stress have been reported in Arabidopsis in a study carried out by Rentsch, et al. [51] and *Reaumuria soongorica* in the study of Liu, et al. [52]. Hence, *Vradi06g04840* may serve as adaptive strategy for the regulation of proline distribution throughout the plant during drought stress.

The candidate gene *Vradi06g03310* annotates for the GDSL esterase genes that are involved in growth, development, and stress responses. Under normal conditions, slight expression was observed but upon drought treatment, high expression level was observed in Arabidopsis and

Soybean [53]. Su, et al. [53] also higher accumulation of proline GDSL esterase transgenic Arabidopsis and Soybean plants under drought stress suggesting a supporting role for proline in GDSL esterase drought tolerance strategy. Another GDSL esterase -type gene, *CaGLIP1*, was also reported by Hong, et al. [54] to be involved in drought stress tolerance in Arabidopsis plant.

The candidate gene *Vradi02g06260* annotates for salicylic acid-binding protein 2-like. Salicylic acid plays an important role in plant growth regulation, development, fruit ripening, and responses to drought, temperature, and salinity stresses. Salicylic acid-binding protein transmits salicylic acid signals during plant stress responses [55]. Low concentration of salicylic acid aid plant growth and development while high concentration induces reactive oxygen species production which leads to reduced tolerance in plants [56]. Studies have reported tolerance of plants to both salinity and water stress in the presence of low concentration of salicylic acid [57,58].

6.4 Conclusion

Being the first report on GWAS analysis of water stress response on BGN, these results represent a major step forward in identifying molecular responses of BGN to water stress and subsequent breeding for improved water stress tolerance in BGN. The results will provide valuable molecular markers and candidate genes for improved BGN breeding. Upon the availability of BGN whole genome sequence, future research should incorporate genomics studies with other omics studies for effective characterization of mechanisms of tolerance and validate genes responsible for controlling these complex traits in BGN.

6.5 Materials and Methods

6.5.1 Plant materials

In this study a set of 95 accessions of the BGN germplasm that was domiciled at the Genetic Resources Center, IITA, Ibadan, Nigeria (Appendix). From the germplasm available in the gene bank, the accessions that have not been previously genotyped were selected for this study. This was done to avoid data duplication.

6.5.2 Experimental set-up and phenotyping

All 95 accessions were planted on 25 October, 2019 in a wooden box in the screen house at IITA, Ibadan. Each wooden box had 5 accessions. The experimental design consisted of randomized complete blocks with three replications; each replication consisted of 19 wooden boxes. After drying and sieving, a soil mixture of 5:1 sandy soil and topsoil was used. All boxes were filled with 32kg of the soil mixture and were watered to field capacity; excess water was allowed to drain for 24 hrs before planting. Each accession was planted with two seeds that were visually selected for having a similar size and good quality. Each box was watered with 500 mL using a graduated cylinder every two days after BGN emergence and then watered with 1000 mL at a one-leaf stage every two days. All seedlings were thinned to one plant per box at approximately 14 days post-planting, and watering was stopped when the plants were at the early vegetative stage. This allowed the plants to be well established before being subjected to stress treatment. The leaf chlorophyll content (CHLCON) of individual plants was measured by using a leaf chlorophyll meter (LC-502, Soil-Plant Analysis Development (LC) Section, Minolta Camera Co., Osaka, Japan). Also, the volumetric water content (VWC) of the soil in each box was measured at the initiation of drought treatment, and every two days throughout drought imposition. Individual plants were scored for wilting, stem greenness, and leaf senescence. Scoring was done on days 7, 10, and 13 before

watering was resumed. Stem greenness was scored on a scale of 0 to 5, with 0 being completely yellow and 5 being completely green. Wilting was scored on a scale of 0 to 5, with 0 being no sign of wilting and 5 being completely wilted. At the termination of the experiment surviving plants were given a score of 1 when recovery occurred and 0 when no recovery was observed. Boxes were watered to field capacity on the day irrigation was resumed, thereafter once every two days for two weeks. The duration of the experiment was nine weeks.

6.5.4 Statistical analysis

Data were analyzed using the statistical package R[59]. Analysis of variance (ANOVA) was performed using the *lmer* package to determine the effect of accessions, the period of stress, and the interaction between accessions and the period of stress on the scored traits. Fischer's least significant difference (F-LSD) was used at a probability level of 5% to separate the mean that were significantly different. Principal component analysis (PCA) was done using the FactoMineR package [60] and Pearson correlation was performed using the cor function of the stats package in R. A hierarchical cluster analysis was performed using the ward.D2 method in a cluster R package [61].

6.5.3 DNA extraction and genotyping

Genomic DNA was extracted from 15-day-old plants using the CTAB protocol [62]. The quality and quantity of all DNA samples extracted were verified on the Nanodrop spectrophotometer and further confirmed on 1% agarose gel run in TAE buffer at 100 V. A total of 93 high-quality DNA samples (50 µL of 100 ng μL^{-1}) were sent to Diversity Array Technology (http://www.diversityarrays.com), Canberra, Australia, to generate SNP markers. SNP markers were generated using the next-generation high-throughput DArTSeq approach, which represents a combination of both complexity reduction and restriction enzymes method [63]. The sequence generated in the FASTQ files was further processed using proprietary DArT analytical pipelines.

The sequences were mapped to the Mungbean reference genome [28] and SNPs were called using the GAPIT pipeline [64].

6.5.4 Genetic diversity and association analysis

Of the 95 accessions, 93 were used in the GWAS studies. The total number of SNP markers used for the GWAS analysis is 5,385. PCA and neighbor-joining tree were created to validate population stratification using the integrated Genomic Association and Prediction Tool (GAPIT) [65]. Association analysis was performed using generalized linear model (GLM) and the mixed linear model (MLM) implemented by GAPIT in R [65] which took into account a K-PC model [66]. Kinship information was included for GWAS, together with the first three principal components (PC) as covariates, which further improves statistical power. Kinship matrix was calculated using the method described by VanRaden [66]. The best fit of the model was evaluated on the Q-Q plots generated by the model. The significant threshold for the association was set at P of 1×10^{-3}. Associations with false discovery rate (FDR) adjusted at 10% were used to determine the P-value thresholds. For any SNPs significantly associated with any of the traits, we performed a candidate gene search within ±100 kb around the locus using annotated genes for the Mungbean genome from Legume Information System (LIS) at https://legumeinfo.org/genomes and the Ensembl genome database [67].

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6.7 Acknowledgments

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6.8 Author contributions

Oluwaseyi Samuel Olanrewaju: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Olaniyi Oyatomi:** Conceptualization, Writing - review & editing, Supervision. **Olubukola Oluranti Babalola:** Writing - review & editing, Supervision. **Michael Abberton:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

6.9 Declaration of Competing Interest

The authors declare no conflict of interest.

6.10 Figure legends

Figure 1. Traits' summary statistics for each environment. An environment represents days after stress imposition.

Figure 2. Biplot and scree plot visualization. (a). Biplot at indicated by different colors for the days after stress was induced. (b). Scree plot showing the contributions of the dimensions to the biplot.

Figure 3. Clustering and correlation analysis (a) Clustering using Ward.D method to group the accessions based on the stress response traits (b) Correlation matrix showing the relationship among the variables throughout the duration of water stress.

Figure 4. PCA and scree plot showing distribution of the components. (a) three-dimensional plot of the distribution of the accessions along the first three principal components (b) scree plot depicting the number of significant PCs.

Figure 5. clustering and heatmap (a) clustering of the accessions based on the SNP panels using neighbor joining method. (b) heatmap of pairwise kinship matrix of the 93 accessions.

Figure 6. SNP distributions and Manhattan plots (a) Significant SNP distribution on each chromosome by both models for each trait at $p < 0.001$. (b) Manhattan plots and Q-Q plots for the traits observed in the study.

6.11 Table legends

Table 1. Summary statistics of the traits scored across all environments

Table 2. ANOVA for the performance of the accessions in response to water stress.

Table 3. Contributions of traits to the principal components' axis.

Table 4. SNPs associated with water stress response traits at a significant level of 0.001.

Table 5. Putative candidate genes and their annotations. Candidate genes were chosen when the entire sequence overlapped with a marker sequence of highly significant QTLs. Physical position on the chromosome, the interval of the genes and their annotation are given.

CHAPTER 7

General discussion, conclusion, and recommendations

7.1 General discussion

The dependence of the global food supply on major crops is a concern for future food availability. These crops alone may not be able to sustain the estimated number of people on the planet, which will exceed 9 billion by 2050 (Gerten et al., 2020, McKenzie and Williams, 2015). It is therefore predicted that an increase in crop yield of around 70% is needed to feed these projected numbers of people (Fischer, 2009). In addition, climate change has affected crop yields negatively due to changes in temperature and rainfall pattern.

Plant genetic resources offer a wide range of diversities that can help to improve food and nutrition security as well as tolerance against adverse conditions such as drought and high temperatures, which are increasing because of climate change (Heller et al., 1995). Most of the genetic diversity in crops is very under-used, while some of it has not been used at all. Only a subset of people in localities where these crops are grown enjoy the benefits from them. Such crops lack scientific insights into their development as they are under-researched (Varshney et al., 2012), so their potential is not fully exploited and they are generally referred to as underutilized or orphan crops. Bambara groundnut (BGN) is one such underutilized crop cultivated in Africa and parts of Asia. This crop harbors a large genetic diversity (Mayes et al., 2019) which is yet to be utilized because there is less interest in research on it compared with the other major crops.

Application of conventional and molecular breeding is important in the improvement of drought tolerance, pest resistance, salt tolerance, increased yield, and other traits in crops. With many germplasm collections and the availability of GBS (through NGS), it is possible to study the phenotypic and molecular responses of crops to different environments and different climatic

conditions. Genomic regions controlling various responses can be identified, gene expression can be studied, and ultimately, better crop varieties can be developed through MAS.

In this study, we assessed the genetic diversity of the selected accessions of BGN in terms of response to the environment, yield and yield-stability, and the nutrient and antinutrient composition. Furthermore, we characterized the responses of the accessions to water stress using the GWAS method.

This study hypothesizes that accessions of BGN are diverse in agronomic performance, physiological traits, nutrient, and antinutrient components. It was further hypothesized that the BGN accessions used are diverse in their responses to drought stress. To test these hypotheses, conventional field experimentation and screen house approaches were adopted. These included experiments evaluating the effect of the environment on growth and yield traits, stability in various environments, nutrient and antinutrient components, and physiological and molecular responses to water stress.

All accessions showed a high level of diversity for all traits studied, supporting the results of high variability in BGN reported in other studies (Gbaguidi et al., 2018, Mohammed et al., 2016, Atoyebi et al., 2017) (Chapter 3). The high coefficient of variation observed showed a high level of heterogeneity among the accessions. Significance in the genotype-environment interactions for all traits showed the impact of the environment and the genetic make-up of the crop on its phenotypic traits. The traits were highly correlated with each other. However, not all correlations were statistically significant. This study shows that locations affect the performance of each accession in the various response traits. Further means of separation of the accessions showed a high level of diversity among the accessions in response to the traits.

MET is useful in selecting the best environmental conditions and the best plant genotypes in those environments. In this study, we were able to determine the best environment for BGN cultivation and the best BGN accessions in the various environments studied (Chapter 4). Biplot analysis, discriminativeness and stability analysis were used, among others. In the pooled ANOVA for the yield, environment, genotype, and interactions were all significant, meaning that both genotype and environment determine the yield of each accession. The Eberhart and Russell method for stability analysis showed the most stable accession in each environment and the most stable environment. This study, with additional research involving more accessions, will help farmers to determine the best time and environment to get the best yield output since yield is the trait most important to farmers. Among the accessions, TVSu-1943 was the highest yielding accession in all test environments, meaning that it can be suggested to farmers who need high yielding accessions. However, in terms of the most ideal accessions, TVSu-2020 and TVSu-1649 are the ideal accessions and IB2019 is the ideal environment.

The importance of nutrients in our diets cannot be overemphasized. However, not all crops have the required amount of nutrients that are needed for the body to function properly. But in BGN, there are sufficient quantities of nutrients that meet the nutrient needs for health. On the other hand, BGN also possesses some antinutrient components that are not good for human health. In Chapter 5 of this study, we were able to show the amount of each nutrient and anti-nutrient component in the selected accessions as well as the relationship between them. The accessions were highly significant for traits and the means separation showed a high level of diversity in the nutrient and antinutrient compositions in the accessions. Cluster, correlation, and PCA analysis were able to provide more information on the level of diversity among the accessions.

As a drought-tolerant crop, BGN is important for food security in sub-Saharan Africa and parts of Asia where it is consumed. In Chapter 6, the different accessions studied responded differently based on the duration of the stress. This shows a high level of variations among accessions and diversity in their responses. By the end of the experiment, only a few accessions were able to recover fully. Chlorophyll content regulates photosynthesis, hence its importance in stress adaptation. This chapter reports a positive, significant correlation between the chlorophyll content of the accessions and their recovery. Trait associations need to be studied and molecular markers detected for improved breeding as a move towards further improvement and positioning of the crop as a viable complement to the major crops. Those genomic regions associated with chlorophyll content, leaf wilting, stem greenness, leaf senescence, and recovery were reported and the chromosome locations of these traits were identified. Different accessions responded to stress in different ways and through different mechanisms, showing diversity in plant responses. Putative candidate genes regulating these responses were identified using the Arabidopsis database and appropriate literature searches.

7.2 Conclusions

In conclusion, we identified large variabilities among the accessions for the traits scored. This shows that these accessions can be a source of genetic diversities for the improvement of this crop. We were also able to determine the best environment from among our "selected" environments, allowing farmers to choose the best environment for planting. The most stable and adaptable accessions were also identified, meaning that these can be a basis for improved breeding programs. The genetic positions on the chromosome controlling water stress response traits scored were analyzed through GWAS and the putative candidate genes regulating these water stress response traits were identified.

Despite its drought tolerance, resistance to pests and diseases, and adaptation to nutrient-poor soils, BGN production is still low and its yield is unpredictable. Therefore, developing high-yielding and adapted accessions will aid the course of BGN in improving food security. From this study, results will be useful in improving BGN cultivation generally, and specifically, as follows.

- It will help to identify the important traits in BGN that can help in alleviating food and nutrition insecurity.
- It will increase the importance of multilocational trials in crop breeding, which will help in selecting the best environment.
- As one of the key goals of the SDG, the nutritional importance of the crop was further established.
- Although BGN is just entering the genomic era, the identification of SNPs controlling the water stress response can be the foundation and catalyst needed for the crop's potential to be fully identified and utilized. No study has yet reported the molecular aspect of the crop's response to water stress.

7.3 Recommendations

- In subsequent METs of the crop, many environments should be considered to capture a complete environmental response.
- Many accessions should also be studied to cover a wide range of diversities.
- Accessions should be assessed for their performance in various environments. Accurate records of exact maturity dates should also be taken so that the pods will not overstay in the soil before they are harvested.
- Due to the GEI effect on the traits, most yield components have low heritable variation. Therefore, conventional breeding alone will not give much improvement in the yield of the

crop. The application of biotechnological tools such as genomics, transcriptomics, and proteomics will aid in gene mapping and responses that could help improve the crop.

- Apart from institutions, researchers hardly ever get funds for this crop. This limits the depth and amount of research on the crop. More funding is needed for this crop to be fully developed and adequately utilized.

- Postharvest procedures such as threshing and storage pose another great challenge. There is no mechanized way of threshing, but when this process is done manually, most of the seeds are destroyed. A large portion of the harvest is lost during threshing. Finding better ways to prevent these big losses will be a big boost for output.

- A comprehensive survey of genetic diversity in landraces and wild relatives of BGN will deepen our understanding of the genetic basis underlying domestication and evolution of this underutilized orphan crop. The use of the NGS technology and informatics combined with information on genetic variation will provide the push needed for BGN to catch up with other crops in studies on functional genomics. The time for genomics-assisted BGN breeding is finally here.

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APPENDIX A

Plate 1: Planting peg and barcoded label

Plate 2: Field marking and seed planting

Plate 3: Bambara groundnut plants on the field

Plate 4: Mature pods

Plate 5: Harvesting of Bambara groundnut pods manually using a hoe

Plate 6: Box layout for water stress experiment

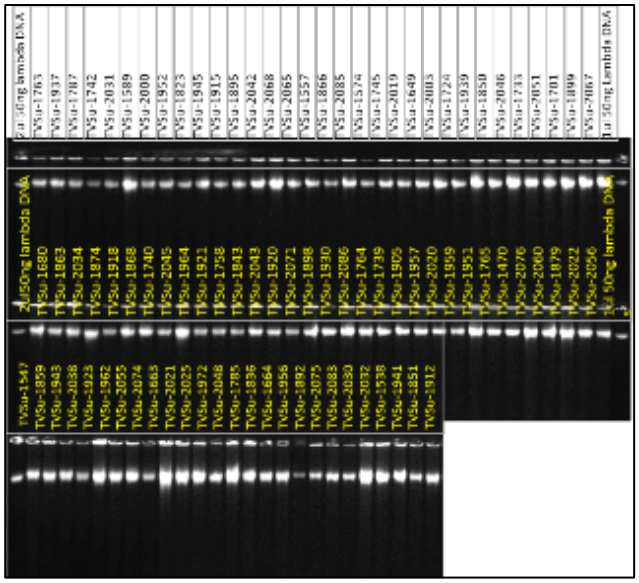


Plate 7: Gel picture for gel electrophoresis of the DNA from the accessions of BGN

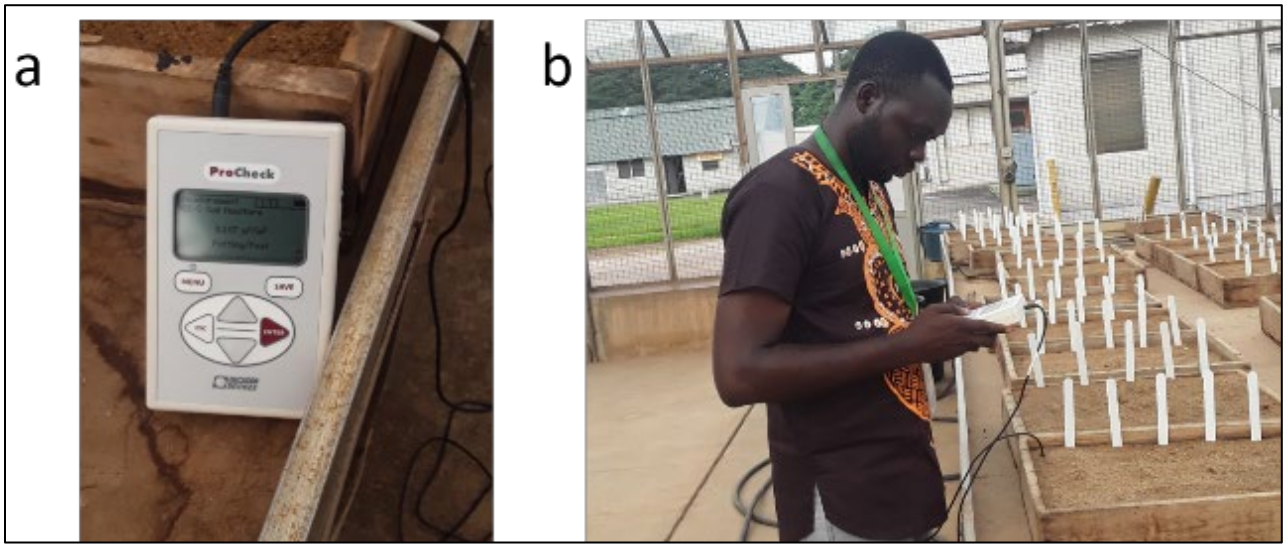
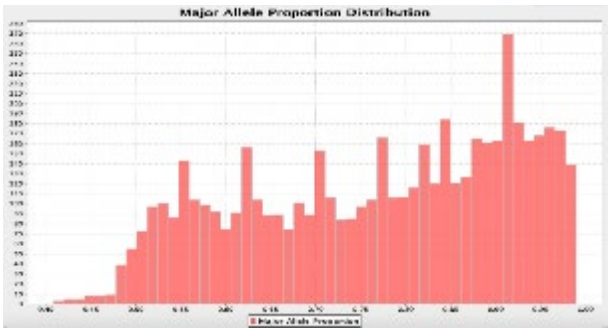
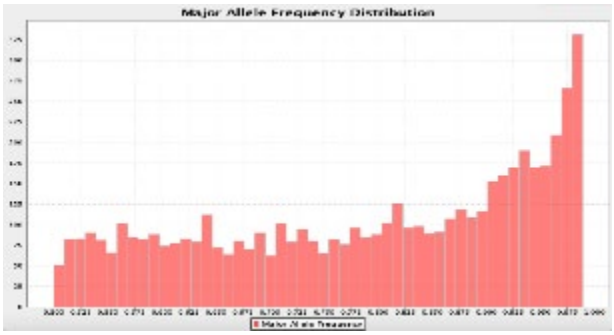
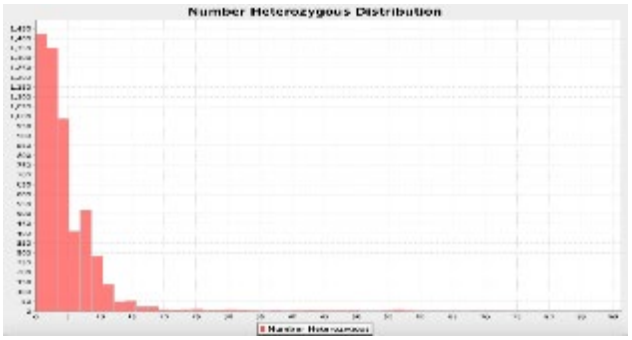


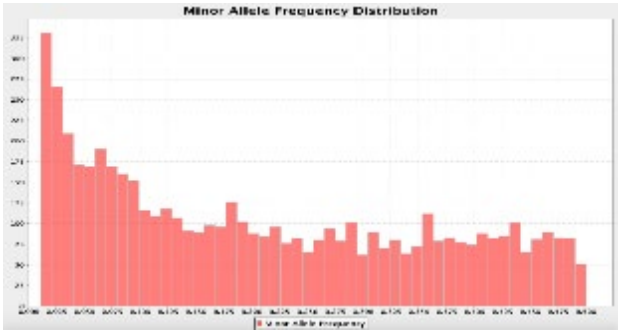
Plate 8: a. Procheck equipment for checking the water level in the box b. Using the procheck equipment

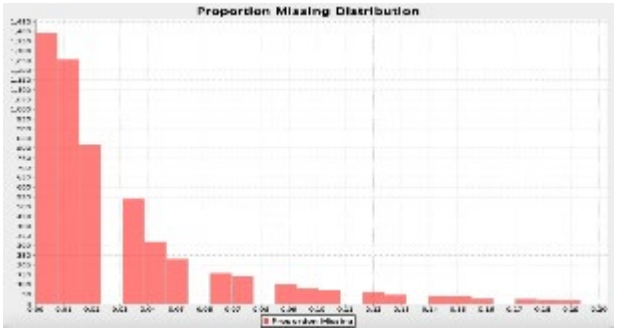
Plate 9: Responses of the plants at various stages of the experiment.

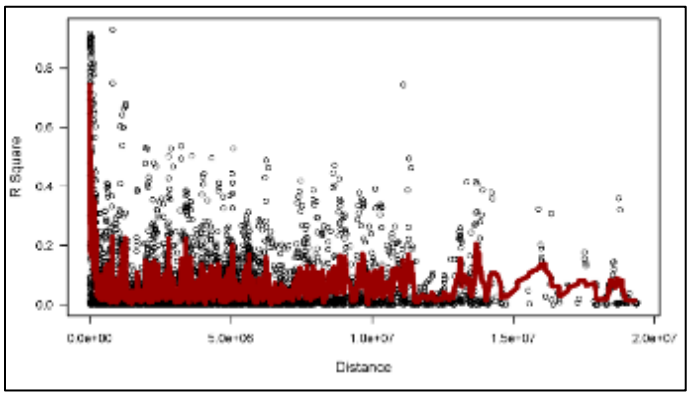












Linkage disequilibrium decay plot of the association mapping panel plotted against physical distance and coefficient of determination (R square).

APPENDIX B

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Frontiers in Plant Science: https://www.frontiersin.org/about/author-guidelines

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Manuscript title: Genetic diversity and environmental influence on growth and yield parameters of Bambara groundnut
Journal: Frontiers in Plant Science, section Plant Breeding
Article type: Original Research
Authors: Oluwaseyi Samuel Olanrewaju, Olaniyi Ajewole Oyatomi, Olubukola Oluranti Babalola, Michael Abberton
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APPENDIX E

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Corresponding Author: Professor Michael Abberton
Co-Authors: Oluwaseyi Samuel Olanrewaju; Olaniyi Oyatomi; Olubukola Oluranti Babalola
Manuscript Number:

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Ref: Submission ID 7a68d377-3ad6-430a-86b0-deb1d7321b89

Dear Dr Olanrewaju,

Please note that you are listed as a co-author on the manuscript "Molecular dissection of early vegetative stage Bambara groundnut drought tolerance using a genome-wide association analysis method", which was submitted to Scientific Reports on 26 November 2021 UTC.

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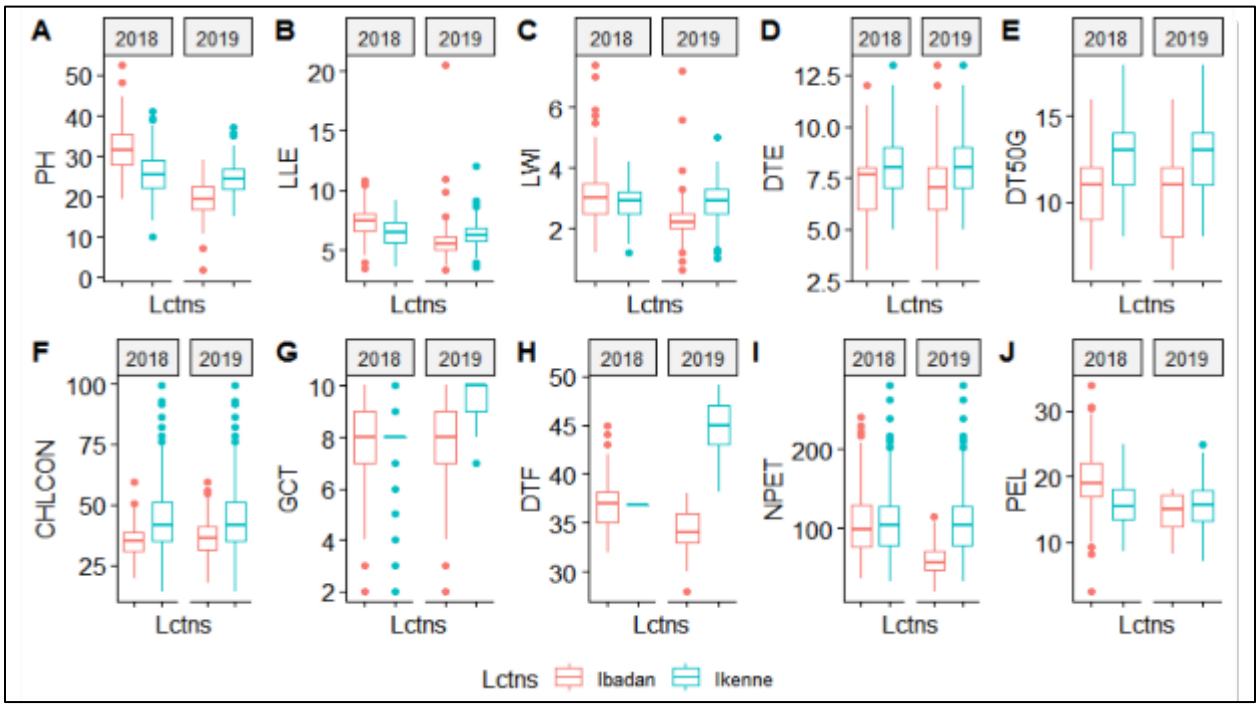


Figure S1|Boxplot summarizing the vegetative growth parameters in the two locations

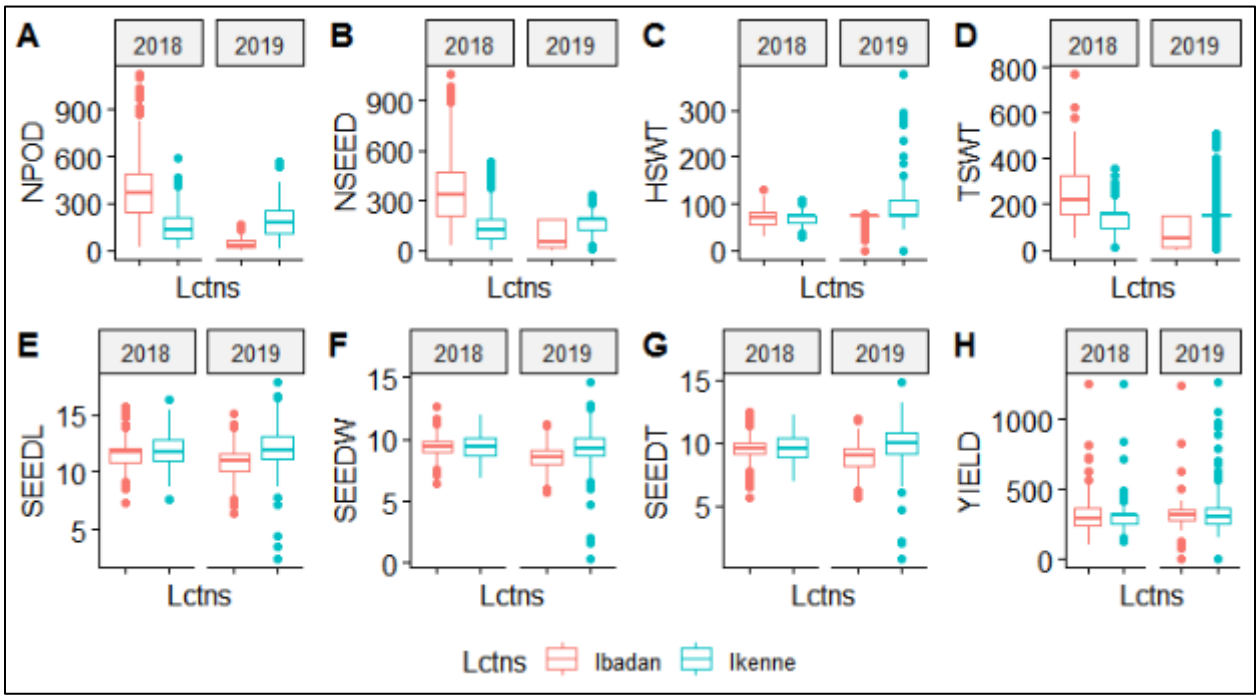
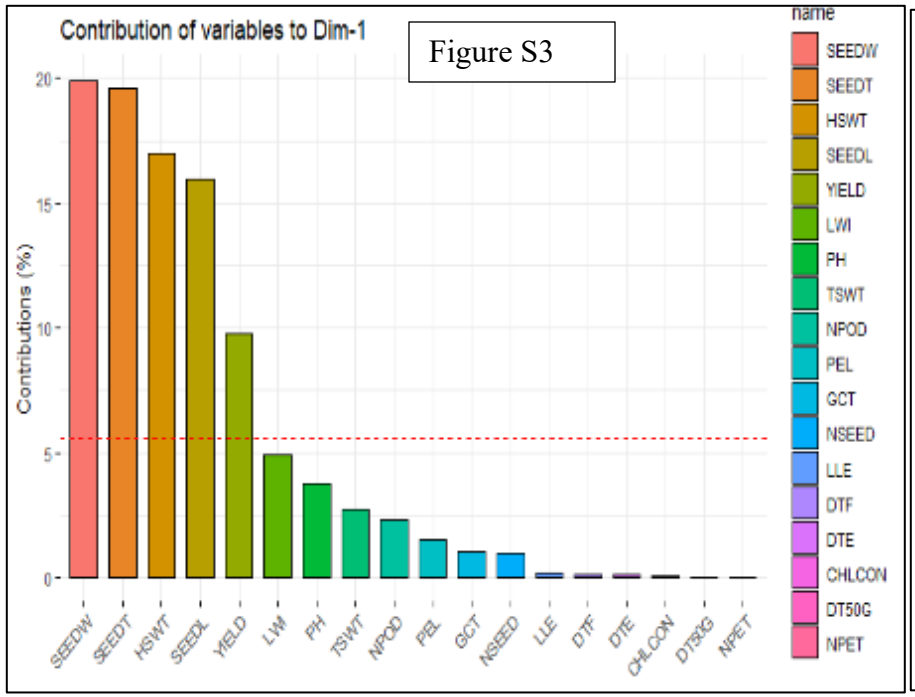


Figure S2|Boxplot summarizing the yield and yield traits in the two locations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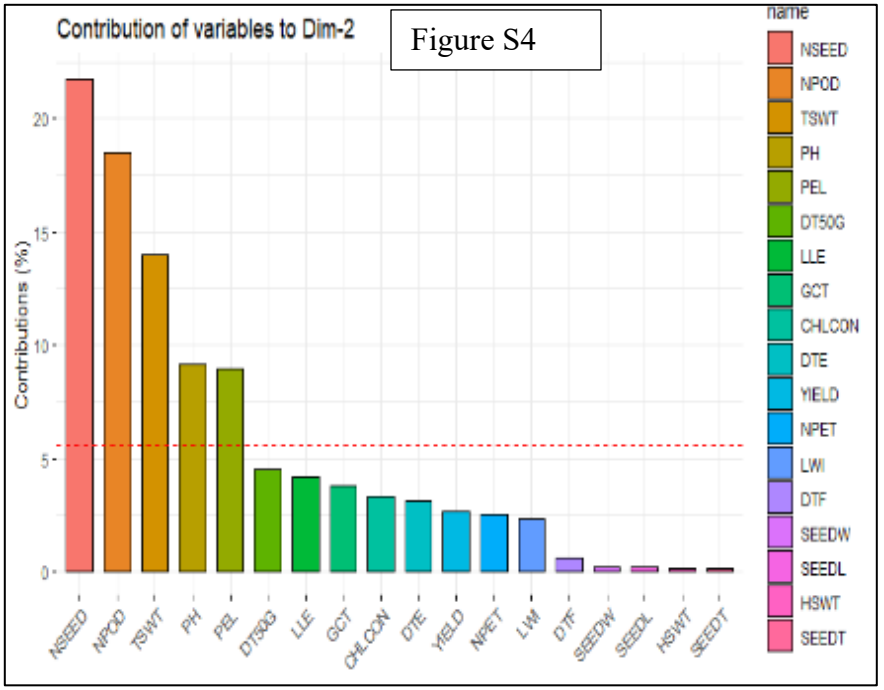
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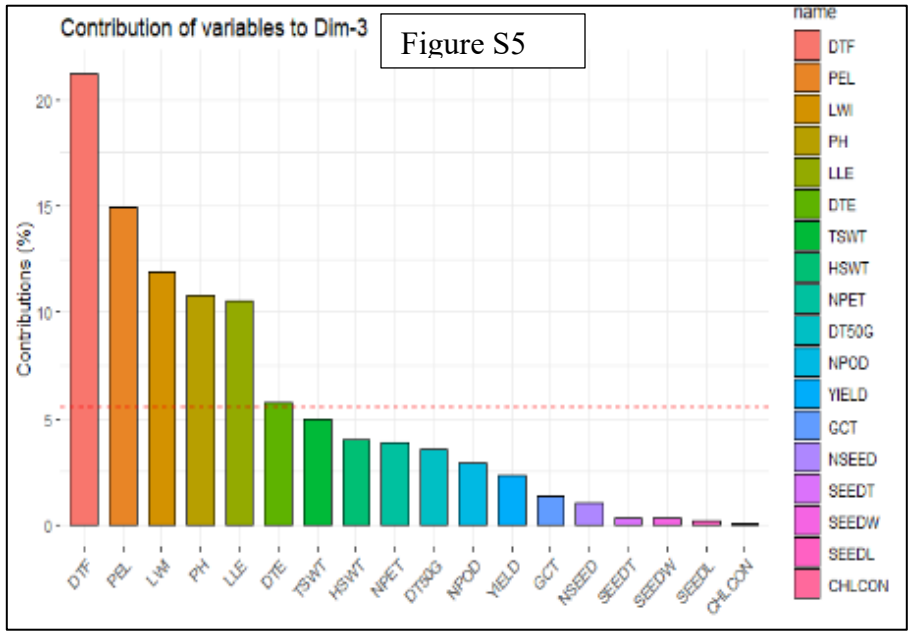
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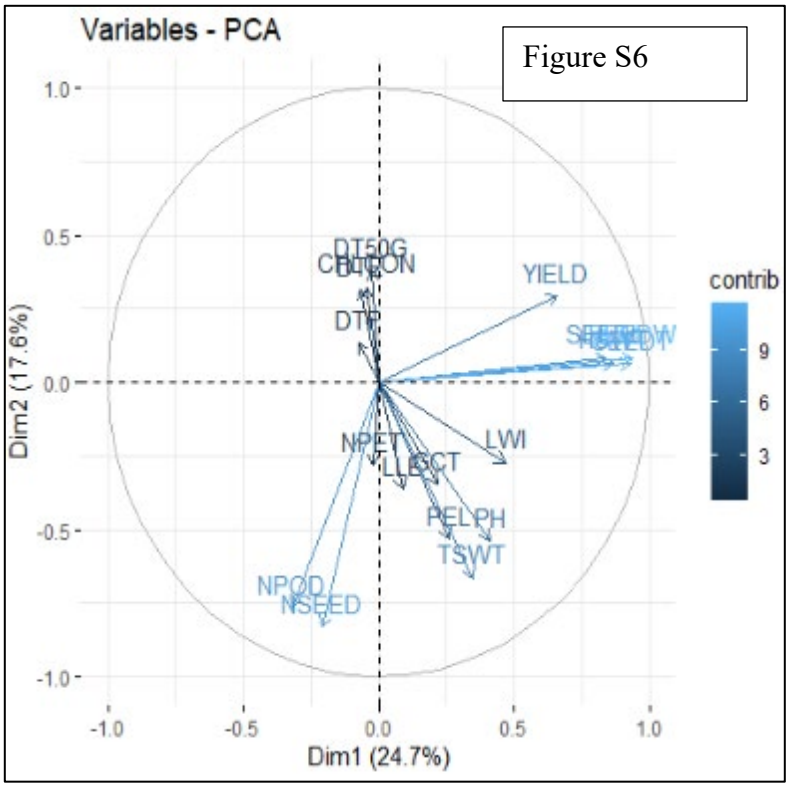
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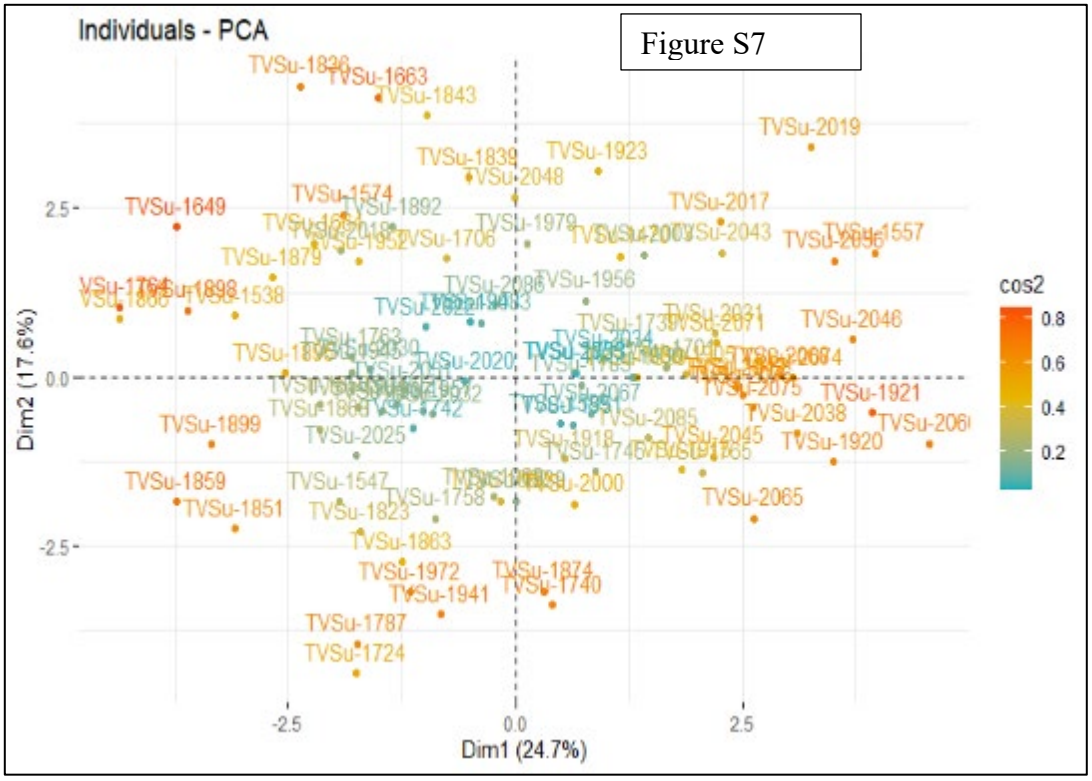
Dim2 (17.6%)
1.0
0.5
0.0
-0.5
-1.0


Individuals - PCA
Figure S7
TVSu-1826
TVSu-1663
TVSu-1843
TVSu-2019
TVSu-1839
TVSu-1923
TVSu-2048
TVSu-1574
TVSu-1649
TVSu-1892
TVSu-2017
TVSu-1879
TVSu-1706
TVSu-1557
TVSu-1956
TVSu-1538
TVSu-2046
TVSu-2020
TVSu-2075
TVSu-1921
TVSu-1899
TVSu-2025
TVSu-2038
TVSu-1920
TVSu-1859
TVSu-1547
TVSu-2065
TVSu-1851
TVSu-1823
TVSu-1863
TVSu-1972
TVSu-1874
TVSu-1941
TVSu-1740
TVSu-1787
TVSu-1724
cos2
0.8
0.6
0.4
0.2
Dim1 (24.7%)
Dim2 (17.6%)
2.5
0.0
-2.5

APPENDIX H

Bambara groundnut accessions and their origin.

	Accessions	Passport data		Accessions	Passport data		Accessions	Passport data
1	TVSu-1470	Ghana	33	TVSu-1866	Zimbabwe	65	TVSu-2017	Burundi
2	TVSu-1538	unknown	34	TVSu-1868	Zimbabwe	66	TVSu-2018	Burundi
3	TVSu-1547	unknown	35	TVSu-1874	Botswana	67	TVSu-2019	Burundi
4	TVSu-1557	unknown	36	TVSu-1879	Botswana	68	TVSu-2020	unknown
5	TVSu-1574	unknown	37	TVSu-1892	Botswana	69	TVSu-2021	unknown
6	TVSu-1589	unknown	38	TVSu-1895	Botswana	70	TVSu-2022	unknown
7	TVSu-1649	Senegal	39	TVSu-1898	unknown	71	TVSu-2025	unknown
8	TVSu-1663	Senegal	40	TVSu-1899	unknown	72	TVSu-2030	unknown
9	TVSu-1664	Senegal	41	TVSu-1905	unknown	73	TVSu-2031	unknown
10	TVSu-1680	Togo	42	TVSu-1912	Cameroon	74	TVSu-2032	unknown
11	TVSu-1701	Togo	43	TVSu-1915	Cameroon	75	TVSu-2034	unknown
12	TVSu-1706	Zambia	44	TVSu-1918	Cameroon	76	TVSu-2038	unknown
13	TVSu-1724	Zambia	45	TVSu-1920	Senegal	77	TVSu-2042	unknown
14	TVSu-1733	Zambia	46	TVSu-1921	Malawi	78	TVSu-2043	unknown
15	TVSu-1739	Zambia	47	TVSu-1923	Zimbabwe	79	TVSu-2045	unknown
16	TVSu-1740	Zambia	48	TVSu-1930	Zimbabwe	80	TVSu-2046	unknown
17	TVSu-1742	Zambia	49	TVSu-1937	Zimbabwe	81	TVSu-2048	unknown
18	TVSu-1745	Malawi	50	TVSu-1939	Zimbabwe	82	TVSu-2051	unknown
19	TVSu-1758	Malawi	51	TVSu-1941	Zimbabwe	83	TVSu-2055	unknown
20	TVSu-1763	Malawi	52	TVSu-1943	Zimbabwe	84	TVSu-2056	unknown
21	TVSu-1764	Malawi	53	TVSu-1945	Zimbabwe	85	TVSu-2060	unknown
22	TVSu-1765	Malawi	54	TVSu-1951	Zimbabwe	86	TVSu-2065	unknown
23	TVSu-1785	Malawi	55	TVSu-1952	Zimbabwe	87	TVSu-2067	unknown
24	TVSu-1787	Cameroon	56	TVSu-1956	Zimbabwe	88	TVSu-2068	unknown
25	TVSu-1823	Niger	57	TVSu-1957	Zimbabwe	89	TVSu-2071	unknown
26	TVSu-1836	Niger	58	TVSu-1959	Swaziland	90	TVSu-2074	unknown
27	TVSu-1839	Zimbabwe	59	TVSu-1962	Swaziland	91	TVSu-2075	unknown
28	TVSu-1843	Zimbabwe	60	TVSu-1964	DRC	92	TVSu-2076	unknown
29	TVSu-1850	Zimbabwe	61	TVSu-1972	DRC	93	TVSu-2083	unknown
30	TVSu-1851	Zimbabwe	62	TVSu-1979	Burundi	94	TVSu-2085	unknown
31	TVSu-1859	Zimbabwe	63	TVSu-2000	Burundi	95	TVSu-2086	unknown
32	TVSu-1863	Zimbabwe	64	TVSu-2003	Burundi			

Mean±standard deviation of the responses of each trait on the accessions.

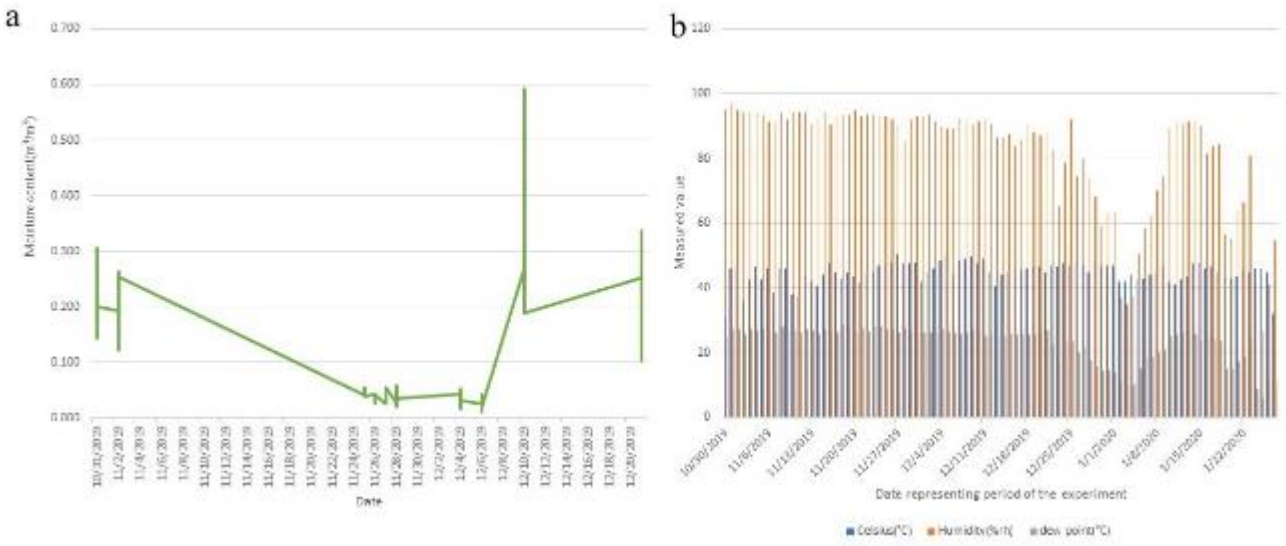
Accns	CHLCON	WTN	LS	SG	Rec
TVSu-1470	33.244±12.29^{g-p}	3.222±1.09^{a-g}	0.889±0.6^{b-f}	2.111±2.37^{b-f}	0.111±0.33^{cd}
TVSu-1538	31.122±10.57^{j-p}	3.556±1.33^{a-d}	1.556±0.73^{a}	2.889±2.26^{abc}	0.111±0.33^{cd}

TVSu-1547	33.056±9.01 g-p	2±1.22 jk	0.444±0.53 fg	1.667±2.5 ef	0.333±0.5 a-d
TVSu-1557	41.522±14.44 a-h	2.889±0.93 b-j	0.667±0.5 d-g	2±2.12 b-f	0.444±0.53 abc
TVSu-1574	38.033±11.5 a-m	2.778±0.97 c-k	0.556±0.53 efg	1.889±2.37 c-f	0.444±0.53 abc
TVSu-1589	33.944±12.52 e-p	2.667±1.32 d-k	1.333±0.71 abc	2.333±2.4 a-f	0.333±0.5 a-d
TVSu-1649	35.056±14.65 b-p	3.111±1.05 a-h	0.667±0.71 d-g	2±2.45 b-f	0.444±0.53 abc
TVSu-1663	35.1±15.52 b-p	3.333±1.22 a-f	1±1 a-f	2.667±2.55 a-e	0.333±0.5 a-d
TVSu-1664	30.633±8.38 k-p	3.222±1.3 a-g	1±0.87 a-f	2.333±2.35 a-f	0.111±0.33 cd
TVSu-1680	36.311±18.04 a-o	3.778±1.09 ab	1.111±0.78 a-e	2.444±2.4 a-e	0.333±0.5 a-d
TVSu-1701	38.167±13.15 a-m	3±1.12 b-i	1±0.87 a-f	2.222±2.64 a-f	0.222±0.44 bcd
TVSu-1706	32.978±9.35 g-p	2.444±1.67 f-k	0.444±0.53 fg	2.333±2.24 a-f	0.333±0.5 a-d
TVSu-1724	36.767±8.78 a-o	3.333±1.12 a-f	0.889±0.93 b-f	2.444±2.4 a-e	0.111±0.33 cd
TVSu-1733	35.989±10.3 a-p	3±1.5 b-i	0.889±0.78 b-f	2.333±2.35 a-f	0.222±0.44 bcd
TVSu-1739	40.511±12.32 a-i	3.444±1.13 a-e	1.333±0.71 abc	2.778±2.22 a-d	0.222±0.44 bcd
TVSu-1740	34.633±6.29 c-p	3±1.41 b-i	0.667±0.87 d-g	2.111±2.37 b-f	0.444±0.53 abc
TVSu-1742	39.656±13.04 a-k	3.222±0.97 a-g	1.111±0.33 a-e	2.333±2.24 a-f	0.333±0.5 a-d
TVSu-1745	36.889±18.82 a-o	4±1.12 a	1.333±0.87 abc	2.778±2.28 a-d	0.222±0.44 bcd
TVSu-1758	35.689±10.06 b-p	2.333±1.22 g-k	0.556±0.73 efg	1.333±2.18 f	0.444±0.53 abc
TVSu-1763	40.167±20.36 a-j	2.778±1.48 c-k	0.778±0.83 c-g	2±2.45 b-f	0.222±0.44 bcd
TVSu-1764	34.8±12.56 b-p	3.667±0.87 abc	1.222±0.97 a-d	2.778±2.22 a-d	0.222±0.44 bcd
TVSu-1765	35.078±4.5 b-p	2.556±1.51 e-k	0.889±0.78 b-f	2.556±2.51 a-e	0.444±0.53 abc
TVSu-1785	34.678±15.85 c-p	3.667±1.12 abc	1±0.93 a-f	2.111±2.37 b-f	0.444±0.53 abc
TVSu-1787	34.767±11.72 b-p	2.111±1.27 ijk	0.556±0.53 efg	2±2.45 b-f	0.222±0.44 bcd
TVSu-1823	41.467±18.91 a-h	1.889±1.17 k	0.778±0.83 c-g	1.889±2.42 c-f	0.333±0.5 a-d
TVSu-1836	36.956±5.5 a-o	3.444±1.24 a-e	1±1 a-f	2.556±2.46 a-e	0.333±0.5 a-d
TVSu-1839	37.967±13.52 a-m	3.111±1.27 a-h	1±0.87 a-f	2.111±2.37 b-f	0.444±0.53 abc
TVSu-1843	35.733±14.74 b-p	3.667±1.22 abc	1.222±0.83 a-d	3.222±2.44 a	0.222±0.44 bcd
TVSu-1850	32.933±12.15 g-p	2.889±1.27 b-j	0.556±0.53 efg	2.222±2.33 a-f	0.333±0.5 a-d
TVSu-1851	32.533±8.46 h-p	2.333±1.12 g-k	0.222±0.44 g	2±2.45 b-f	0.333±0.5 a-d
TVSu-1859	35.122±13.06 b-p	3.111±1.27 a-h	0.778±0.97 c-g	2.556±2.46 a-e	0.444±0.53 abc
TVSu-1863	31.867±9.2 i-p	2.444±1.01 f-k	0.556±0.53 efg	1.778±2.44 def	0.556±0.53 ab
TVSu-1866	35.933±13.69 a-p	2.111±0.93 ijk	0.222±0.44 g	1.778±2.44 def	0.222±0.44 bcd
TVSu-1868	39.7±21.74 a-k	2.889±1.17 b-j	0.778±0.83 c-g	2.111±2.52 b-f	0.667±0.5 a
TVSu-1874	34.489±6.83 d-p	2.667±1.22 d-k	0.556±0.53 efg	2.222±2.33 a-f	0.222±0.44 bcd
TVSu-1879	36.456±15.79 a-o	2.444±1.13 f-k	0.778±0.44 c-g	1.778±2.44 def	0.333±0.5 a-d
TVSu-1892	40.5±13.6 a-i	2.556±1.13 e-k	0.556±0.73 efg	1.778±2.22 def	0.444±0.53 abc
TVSu-1895	33.6±11.46 g-p	2.111±0.93 ijk	0.556±0.53 efg	1.667±2.5 ef	0.333±0.5 a-d
TVSu-1898	32.6±7.49 h-p	3±1 b-i	0.889±0.33 b-f	2.667±2.4 a-e	0.444±0.53 abc
TVSu-1899	34.611±9.86 c-p	2.778±1.56 c-k	0.778±0.83 c-g	2.444±2.51 a-e	0.333±0.5 a-d
TVSu-1905	35.633±10.51 b-p	3.222±0.97 a-g	1.111±0.78 a-e	2.889±2.26 abc	0.222±0.44 bcd
TVSu-1912	35.756±9.39 b-p	3.111±1.62 a-h	0.889±0.93 b-f	2.667±2.55 a-e	0.222±0.44 bcd
TVSu-1915	26.822±8.45 p	3.333±1.32 a-f	1±1 a-f	2.333±2.4 a-f	0±0 d
TVSu-1918	29.478±8.81 m-p	3.111±1.62 a-h	1±0.71 a-f	2.333±2.55 a-f	0.111±0.33 cd
TVSu-1920	36.3±10.02 a-o	1.889±1.36 k	0.556±0.53 efg	1.778±2.22 def	0.333±0.5 a-d

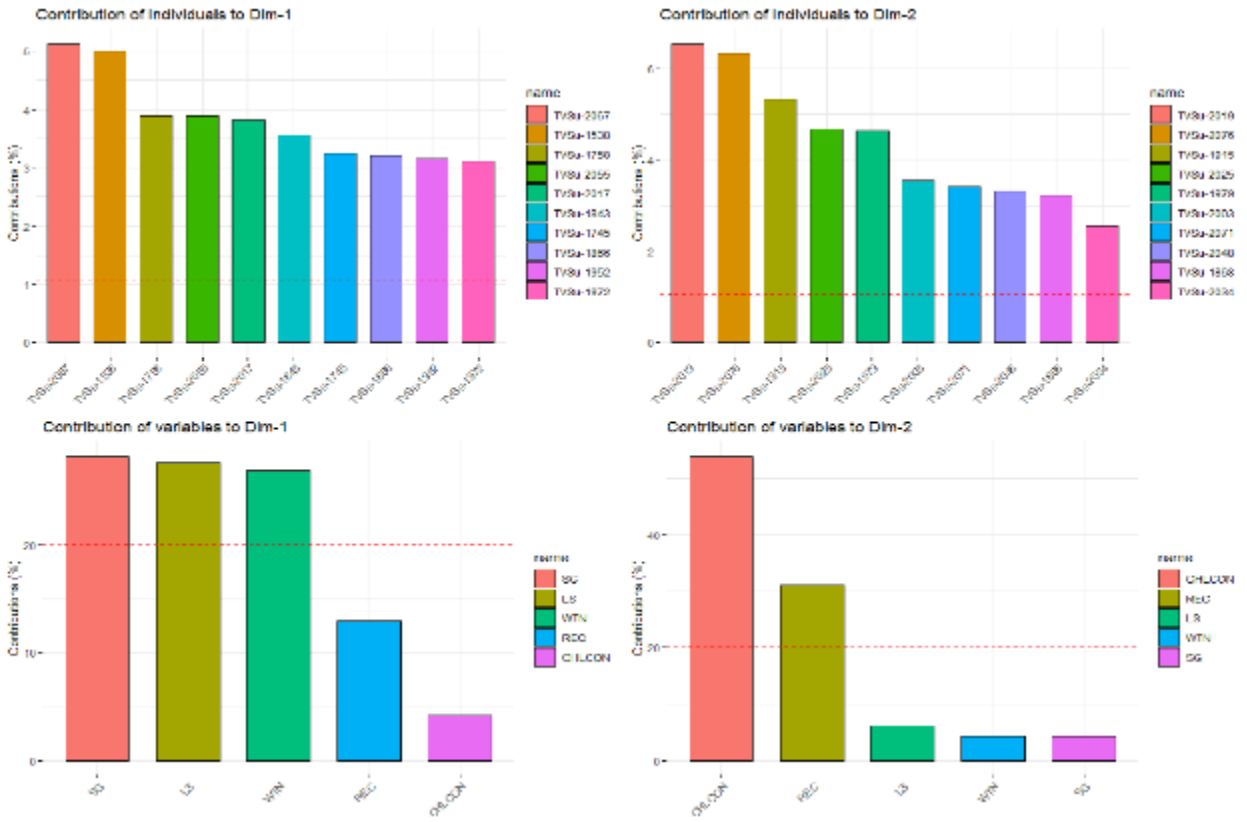
TVSu-1921	35.944±7.38 a-p	3.222±1.39 a-g	1.222±0.83 a-d	2.222±2.44 a-f	0.556±0.53 ab
TVSu-1923	41.744±19.03 a-h	2.889±1.54 b-j	1±0.71 a-f	2.444±2.4 a-e	0.111±0.33 cd
TVSu-1930	35.544±11.11 b-p	2.444±1.59 f-k	0.667±0.87 d-g	1.889±2.42 c-f	0.222±0.44 bcd
TVSu-1937	38±7.25 a-m	2.778±1.56 c-k	1±1 a-f	2.667±2.55 a-e	0.222±0.44 bcd
TVSu-1939	38.244±15.07 a-m	2.778±0.97 c-k	0.889±0.6 b-f	2.111±2.37 b-f	0.444±0.53 abc
TVSu-1941	35.011±6.82 b-p	2.667±1.41 d-k	0.778±0.83 c-g	2.111±2.52 b-f	0.222±0.44 bcd
TVSu-1943	36.133±13.71 a-o	2.667±1.12 d-k	0.556±0.53 efg	1.778±2.44 def	0.333±0.5 a-d
TVSu-1945	34.822±12.69 b-p	3.333±1.41 a-f	0.889±0.93 b-f	1.889±2.42 c-f	0.111±0.33 cd
TVSu-1951	36.478±6.89 a-o	3.444±0.88 a-e	0.889±0.78 b-f	2.556±2.51 a-e	0.222±0.44 bcd
TVSu-1952	36.956±12.61 a-o	3.778±1.2 ab	1.444±0.88 ab	2.778±2.33 a-d	0.222±0.44 bcd
TVSu-1956	34.322±10.75 e-p	3±1.73 b-i	1.111±0.78 a-e	2.667±2.55 a-e	0.111±0.33 cd
TVSu-1957	29.922±9.09 m-p	3.333±1.12 a-f	1±0.87 a-f	2.444±2.4 a-e	0.111±0.33 cd
TVSu-1959	37.978±15.22 a-m	3.111±1.05 a-h	0.667±0.71 d-g	2.333±2.4 a-f	0.222±0.44 bcd
TVSu-1962	34.333±11.05 e-p	2.778±1.56 c-k	1.222±0.83 a-d	2.556±2.3 a-e	0.444±0.53 abc
TVSu-1964	36.067±8.55 a-o	3.111±1.05 a-h	1.111±0.93 a-e	2±2.35 b-f	0.333±0.5 a-d
TVSu-1972	37.122±7.1 a-o	2.556±1.13 e-k	0.667±0.5 d-g	1.667±2.5 ef	0.667±0.5 a
TVSu-1979	45.144±23.79 a	3.222±1.3 a-g	1±1 a-f	2.556±2.46 a-e	0.333±0.5 a-d
TVSu-2000	32.844±7.23 g-p	3±1.58 b-i	1±0.87 a-f	2.111±2.52 b-f	0.222±0.44 bcd
TVSu-2003	43.633±14.36 a-d	3.222±1.72 a-g	1±1 a-f	2.556±2.46 a-e	0.333±0.5 a-d
TVSu-2017	42.867±15.3 a-f	2.556±1.42 e-k	0.444±0.53 fg	1.778±2.11 def	0.556±0.53 ab
TVSu-2018	35.111±11.1 b-p	3.667±1 abc	0.889±0.6 b-f	2.222±2.44 a-f	0.222±0.44 bcd
TVSu-2019	43.9±16.64 ab	3.333±0.71 a-f	1.111±0.93 a-e	2±2.35 b-f	0.556±0.53 ab
TVSu-2020	33.756±12.32 f-p	3.778±1.09 ab	0.778±0.83 c-g	2.556±2.3 a-e	0.111±0.33 cd
TVSu-2021	30.744±12.15 k-p	2.667±1.66 d-k	0.667±0.87 d-g	2.333±2.55 a-f	0.333±0.5 a-d
TVSu-2022	34.878±13.19 b-p	3±1 b-i	1.111±0.6 a-e	2.222±2.28 a-f	0.222±0.44 bcd
TVSu-2025	28.278±8.08 op	3.333±1.5 a-f	1±0.87 a-f	2.111±2.37 b-f	0±0 d
TVSu-2030	33.689±9.5 f-p	3.333±1.32 a-f	1±0.87 a-f	2.556±2.51 a-e	0.444±0.53 abc
TVSu-2031	34.489±9.03 d-p	3.111±1.45 a-h	0.889±0.93 b-f	2.556±2.46 a-e	0.111±0.33 cd
TVSu-2032	34.744±12.89 b-p	2.889±0.93 b-j	0.556±0.53 efg	2±2.45 b-f	0.444±0.53 abc
TVSu-2034	43.711±6.85 abc	2.444±1.67 f-k	0.778±0.83 c-g	2.222±2.64 a-f	0.444±0.53 abc
TVSu-2038	30.633±10.39 k-p	2.556±1.81 e-k	1±0.87 a-f	2.444±2.4 a-e	0.222±0.44 bcd
TVSu-2042	30.911±12.45 k-p	2.889±1.62 b-j	1.111±0.93 a-e	2.556±2.51 a-e	0.111±0.33 cd
TVSu-2043	39.222±13.6 a-l	2.444±1.42 f-k	0.778±0.83 c-g	2±2.24 b-f	0.556±0.53 ab
TVSu-2045	37.856±13.99 a-n	2.889±1.05 b-j	1±0.71 a-f	2.667±2.35 a-e	0.333±0.5 a-d
TVSu-2046	35.589±11.99 b-p	3.222±0.97 a-g	1.111±0.6 a-e	2.444±2.01 a-e	0.333±0.5 a-d
TVSu-2048	42.011±22.15 a-g	3.333±1.5 a-f	1.222±0.97 a-d	2.556±2.46 a-e	0.333±0.5 a-d
TVSu-2051	37.356±13.39 a-o	3±1.12 b-i	1±1 a-f	2.111±2.52 b-f	0.222±0.44 bcd
TVSu-2055	33.067±8.05 g-p	3.667±1.22 abc	1.333±0.87 abc	2.889±2.26 abc	0.111±0.33 cd
TVSu-2056	37.056±15.59 a-o	3±1.41 b-i	1.111±0.78 a-e	2±2.45 b-f	0.556±0.53 ab
TVSu-2060	28.678±10.07 nop	3.222±1.39 a-g	1.333±0.87 abc	2.778±2.64 a-d	0.222±0.44 bcd
TVSu-2065	34.178±5.88 e-p	3.444±1.13 a-e	1.111±0.78 a-e	2.556±2.46 a-e	0.111±0.33 cd
TVSu-2067	30.822±10.43 k-p	3.444±1.81 a-e	1.556±0.88 a	3±2.35 ab	0.111±0.33 cd
TVSu-2068	31.411±11.79 i-p	2.444±1.24 f-k	0.889±0.6 b-f	1.889±2.2 c-f	0.222±0.44 bcd

TVSu-2071	31.633±5.78 $^{i-p}$	2.222±0.83 $^{h-k}$	0.889±0.6 $^{b-f}$	1.889±2.42 $^{c-f}$	0.111±0.33 ^{cd}
TVSu-2074	29.778±6.85 $^{m-p}$	3.444±1.51 $^{a-e}$	1±1 $^{a-f}$	2.333±2.35 $^{a-f}$	0.111±0.33 ^{cd}
TVSu-2075	37.722±8.24 $^{a-n}$	3±1.41 $^{b-i}$	0.556±0.73 ^{efg}	2.444±2.4 $^{a-e}$	0.333±0.5 $^{a-d}$
TVSu-2076	30.267±7.89 $^{l-p}$	2.889±1.36 $^{b-j}$	0.556±0.53 ^{efg}	1.778±2.44 ^{def}	0±0 ^{d}
TVSu-2083	37.367±16.72 $^{a-o}$	3.333±1.32 $^{a-f}$	1.333±0.87 ^{abc}	2.667±2.55 $^{a-e}$	0.333±0.5 $^{a-d}$
TVSu-2085	37.344±18.63 $^{a-o}$	3±1.12 $^{b-i}$	0.556±0.73 ^{efg}	1.778±2.44 ^{def}	0.222±0.44 ^{bcd}
TVSu-2086	43.056±12.06 $^{a-e}$	2.222±0.97 $^{h-k}$	0.556±0.73 ^{efg}	2.222±2.33 $^{a-f}$	0.444±0.53 ^{abc}
Day 7	43.812±13.72 ^{a}	1.972±0.87 ^{c}	0.345±0.5 ^{c}	0.011±0.1 ^{c}	0.611±0.49 ^{a}
Day 10	36.256±7.04 ^{b}	2.905±1.09 ^{b}	0.951±0.82 ^{b}	1.884±1.8 ^{b}	0.211±0.41 ^{b}
Day 13	26.909±8.57 ^{c}	4.039±0.98 ^{a}	1.386±0.61 ^{a}	4.93±0.44 ^{a}	0.06±0.24 ^{c}

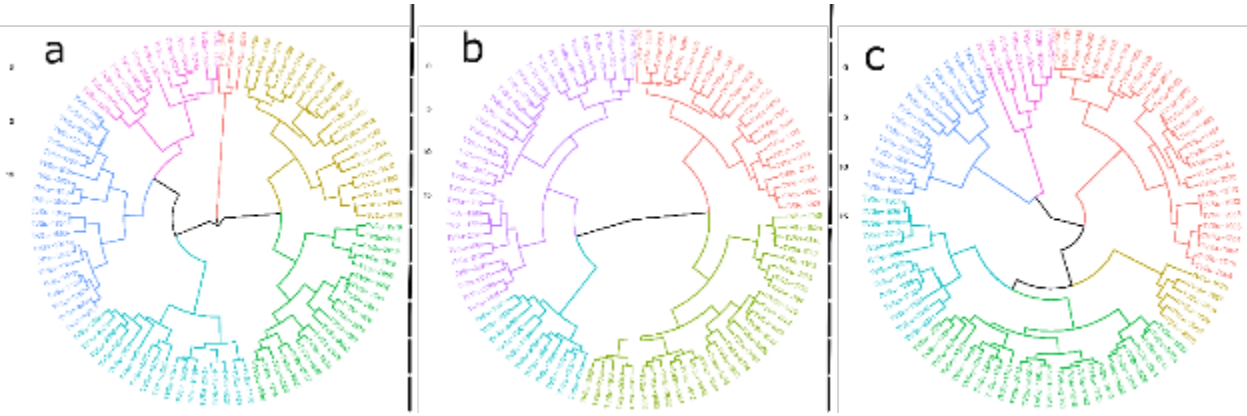
Numbers representing means±standard deviation in a column followed by the same letter are not significantly different according to Fischer's least significant difference (LSD) test ($p<0.05$).



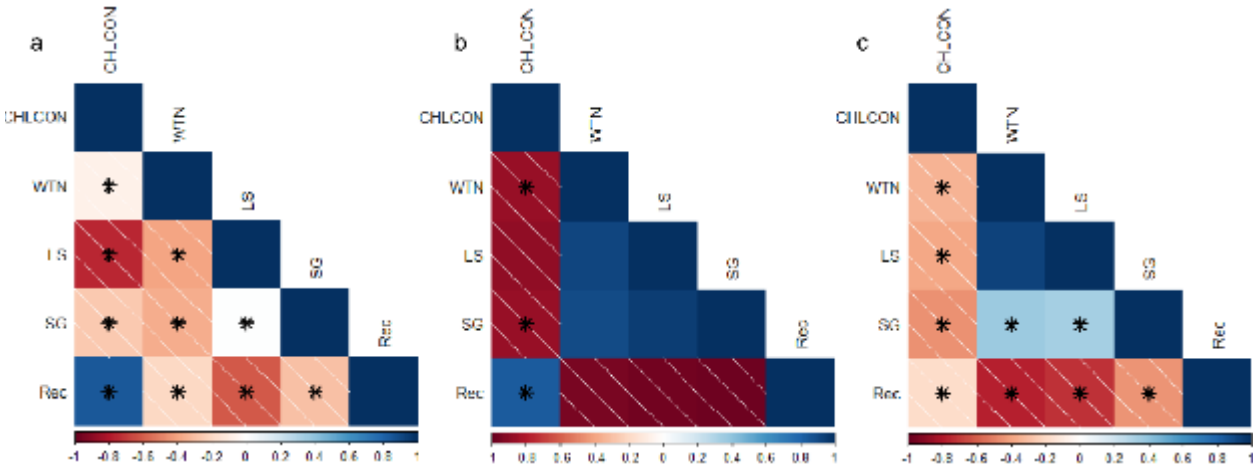
Climatic data of the screen house throughout the period of the water stress experiment.



Contributions of the traits and accessions to the principal components in response to stress treatment



Hierarchical clustering of the trait responses at each period of water stress (a) day 7 (b) day 10, and (c) day 13



Correlation analysis of the trait responses at each period of water stress (a) day 7 (b) day 10, and (c) day 13