ASSESSMENT OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEAN* L. VERDC) RECOMBINANT INBRED LINES (RILS) FOR AGRO-MORPHOLOGICAL TRAITS, COOKING QUALITY PROPERTIES AND NUTRITIONAL COMPOSITION

by

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PREFACE

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

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the reported results are those obtained from the investigations carried out by the candidate.

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DECLARATION

I, Amanda Ruzive, declare that:

i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work.

ii) this dissertation has not been submitted in full, or in part, for any degree or examination to any other university;

iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.

iv) this dissertation does not contain the writing of other persons, unless it is specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

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

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v) where I have used material from which publications have followed, I have indicated my role in the work, in detail.

vi) this dissertation is primarily a collection of the material prepared by me, which has been published as journal articles, or presented as a poster and as oral presentations at conferences. In some cases, additional material has been included; and

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ABSTRACT

Food insecurity and malnutrition are two of the biggest challenges faced by people living in developing countries. Underutilised legumes, such as the bambara groundnut (BGN), are rich in nutrients and can be used to reduce these problems. Although the BGN can be used to reduce food and nutrition insecurity, it remains an underutilised crop because of its hard-to-cook traits. To date, there are no registered BGN varieties in South Africa (SA). This study assessed the agro-morphological diversity of selected BGN genotypes, their cooking quality, as well as the impact of soaking and boiling on their nutritional composition.

This study assessed the agro-morphological diversity of selected BGN genotypes, their cooking quality, as well as the impact of soaking and boiling on their nutritional composition. A total of 346 Recombinant Inbred Lines (RILs), which were developed from four parents (Ankpa4, S19. IITA686 and LunT) and 21 check varieties, were used in the first part of the study. Significant differences ($p<0.05$) were found among some of the traits such as days to emergence, days to flowering, plant height, terminal leaf shape, plant growth habit, canopy width and terminal leaf colour. Furthermore, the correlation analysis showed that the days to emergence, the days to 50% flowering, the plant height and petiole length were positively correlated to the grain yield suggesting the direct selection of these traits in BGN genotypes will be useful for an improved grain yield.

In addition, the second objective was to assess the cooking quality of the BGN genotypes, as well as the impact of soaking and cooking on their nutritional composition. A total of 156 genotypes were used in this study and were selected from the first experiment, based on the grain yield per plot. The cooking time was positively correlated to the electrical conductivity, the degree of lightness, the texture and seed size, but it was negatively correlated to the pH, hydration and swelling properties. There were significant differences in the total mineral content (ash), fat, moisture, NDF and protein content of the raw the raw and cooked BGN samples. The results of this study could help to identify the lines that can be used as parents in the breeding of varieties that cook fast and retain more nutrients after cooking.

Genotype LunT-367-286 showed the highest yield, S19/Ankpa 4-100-87 showed the fastest cooking time and Burkina genotype showed the highest protein content after cooking, which indicates that these genotypes could be used to develop varieties with desirable characteristics in future plant breeding programmes. Overall, the study confirmed the suitability of the BGN for breeding. BGN breeding lines showed a considerable variation in their agro-morphological traits and cooking quality, which suggests that selection can target BGN genotypes with a high yield potential, a short cooking time and a high nutritional content as parental lines for breeding. Further research is recommended on the molecular and sensory characterisation of the genotypes, as well as their consumer acceptability.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
AOAC	-	Association of Analytic Chemists
BGN	-	Bambara Groundnut
CT	-	Cooking Time
CW	-	Canopy Width
DE	-	Days to Emergence
DF	-	Days to 50% Flowering
DW	-	Dry Weight basis
EC	-	Electrical Conductivity
GY	-	Grain Yield
GYP	-	Grain Yield per Plant
HC	-	Hydration Capacity
HI	-	Hydration Index
HTC	-	Hard-to-Cook
IL	-	Internode Length
IPGR	-	International Plant Genetic Resources Institute
NDF	-	Neutral Detergent Fibre
PC	-	Principal Component
PCA	-	Principal Component Analysis
PH	-	Plant Height
PL	-	Petiole Length
R	-	Correlation Coefficient
RCBD	-	Randomised Complete Block Design
RIL	-	Recombinant Inbred Line
SC	-	Swelling Capacity
SI	-	Swelling Index
SS	-	Seed Size
SSA	-	sub-Saharan Africa
SV	-	Seed Volume

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CHAPTER 1
INTRODUCTION, THE PROBLEM, AND ITS SETTING

1.1 Background

The bambara groundnut (BGN) (*Vigna subterranean*) is an important protein source across sub-Saharan Africa (SSA), especially in rural communities where animal protein is relatively expensive. BGN originated in West Africa, but it has become widely distributed throughout the semi-arid regions of SSA. It is drought tolerant, as it can produce higher yields under drought conditions (Mwale et al., 2007; Jorgensen et al.,2010; Chai et al.,2016). However, the legume is one of the most essential, but forgotten and underutilised, crop species. Underutilised crop species with essential nutrients can fit into several niches in food production systems and are better adapted to low farming input systems worldwide (Gruber, 2017). BGN is highly nutritious and provides an alternative, cheaper protein source, compared to animal-based protein. Its seed contains 61-69% carbohydrate, 17-21% protein, 3.3-6.4% fibre, 3.1-4.4% ash and 3.6-7.4% fat, hence it is commonly termed a ‘complete balanced diet’ (Khan et al., 2021). It also contains micronutrients, such as zinc, iron, calcium and potassium, which are important for the immune system, as well as for physical growth and development. Considering its nutritional composition and ability to produce a considerable yield under harsh environmental conditions, BGN is regarded as the crop of the future (Muhammad et al., 2020).

Unlike major crops, such as wheat, rice, maize and potatoes, BGN has been neglected and underutilised. This could be due to the industry itself, international research bodies, as well as the perception of the scientific community that this crop lacks economic importance (Cullis & Kunert, 2017). The hard-to-cook (HTC) phenomenon has been identified as one of the major drawback for the utilisation of BGN (Adzawla et al., 2016). BGN processing in rural areas is carried out at a household level and occasionally as a collaborative practice by women during functions and community activities (de Kock, 2013). However, if BGN is to be considered for large-scale production, research should be conducted on short cooking time varieties to minimise their energy use. In SSA, firewood and charcoal are used as the primary fuel for cooking; they account for more than 80% of the energy consumption (Hailu & Kumsa, 2021), resulting in excessive deforestation developing.

Environmental and storage conditions influence the cooking time of legumes, as indicated by (Garruti & Bourne, 1985; Yousif et al., 2007); however, according to Williams and Singh (1987), the cooking time is also a heritable trait. Many studies have reported that HTC traits are controlled by a few highly heritable genes (Arns et al., 2018; Jacinto-Hernandez et al., 2003). Several hypotheses have been postulated for developing the HTC phenomenon in legumes. The mechanisms responsible for seed hardness include the autolysis of cytoplasmic organelles, the weakling plasma lemma integrity and the lignification of middle and compositional changes, such as the formation of insoluble pectate, lipid oxidation, polymerisation, phytic catabolism, the interaction of proteins, the polymerisation of phenolic compounds and the deposition of lignin-like material (Garcia-Vela & Stanley, 1989; Hincks & Stanley, 1985). The cooking quality of legumes can be evaluated by using the cooking time (Moscoso et al., 1984). A sensory analysis, the tactile method, the spread area ratio, the Mattson bean cooker, the white core method, and the glass slide method are the most common methods that are used to measure the cooking time directly (Wood, 2016). The finger-pressing method has also been widely used to determine the cooking time of grain legumes, as it is simple and cheap (Wainaina et al., 2021).

The management of HTC legumes is not only done to reduce their cooking time but also to improve their nutritional quality. However, some studies have reported that some strategies reduce the availability of nutrients. Mubaiwa et al. (2017) reported that cooking BGN seed with alkaline rock salts (0.5% $NaHCO_3$ and 0.5 gowa or soda water) caused a 13% and 20% reduction in the cooking time, respectively. The same study also identified that both traditional boiling and grit processing improved their *in-vitro* protein digestibility, their *in-vitro* starch digestibility and their mineral bio-accessibility. Rehman et al. (2001) identified a reduction of 2.5% to 13.6% and 7.03% to 28.0% of the total soluble sugars in soaked kidney beans in tap water and sodium bicarbonate, respectively.

The use of a salt solution has been reported to cause a significant reduction in the cooking time of different legumes; however, it is also reported to increase the leaching of solids and pH in cowpeas (Uzogara et al., 1988). Furthermore, Minka et al. (1999) reported the reduction of lipids, neutral sugars and proteins in black beans. In addition, it has also been shown that there is a significant reduction in the protein quality when cooking in a salt solution. The canning of BGNs has also been successfully used to manage its HTC traits; however, these canned products are relatively expensive, especially for people living in rural communities (de Kock, 2013). Plant breeding has been successful in the development of fast-cooking varieties with enhanced nutrition; for example, the Manteca yellow bean (Wiesinger et al., 2018) and the NUA 45, NUA 35 and NUA 50 (Sefume, 2012). These problems can be managed by means of plant breeding; however, the genetics of the cooking time is less understood. Phenotyping is a prerequisite of most plant breeding activities; therefore, it is important to phenotype BGN cultivars and identify the lines with the desired agro-morphological traits, to shorten the cooking time and to retain more nutrients after cooking, to use them for future breeding work.

1.2 Problem Statement

BGN longer cooking time and high energy consumption limit the utilisation of BGNs in most rural communities. The availability of energy in developing countries remains a significant challenge, especially SSA countries, with over 570 million people living without electricity (IEA, 2021). Most of these people use wood fuel, as it is cheaper than Liquefied Petroleum Gas (LPG) and kerosene (Sepp, 2014). In some provinces in South Africa, a large proportion of the people live in rural areas, and they use about 40% of the fuel wood for cooking leading to deforestation and soil erosion (Statistics SA, 2014). Although the consumption of BGNs has great potential for reducing food insecurity and malnutrition in Africa, its utilisation is minimised due to the limited energy supply. The seed hardness trait has a negative impact, in that the cooked seeds may lose nutritional quality, the cost of consumption is increased and germination is uneven (Stanley, 1992). Some cultivars cook faster than others; however, the mechanisms behind these differences are not fully understood, and although the cooking process affects the availability and accessibility of nutrients, the magnitude of these effects have not been fully documented. Despite the supporting evidence in the literature detailing the heritability of the cooking time in legumes, little effort has been made to use this information in the breeding of orphaned crop species, including the BGN.

1.3 Justification

Food insecurity is one of the main factors that worsens malnutrition in rural communities, due to the lack of access to, and the availability of, nutrient-dense foods. The increased consumption of BGNs could contribute towards a reduction in malnutrition; however, its utilisation is limited due to the HTC phenomenon. The management of HTC legumes include chemical treatments (cooking aids), biological treatments (germination and fermentation) and physical treatments (milling, roasting and canning) (Mubaiwa et al., 2017). The goal of legume processing is not only to solve the HTC phenomenon, but also to retain the importance of the sensory and nutritional aspects of legume-based products. However, most of the techniques that are currently used to control the HTC phenomenon result in the reduced nutritional value of the BGN.

Breeding for a short cooking time is a sustainable way of managing HTC BGNs. There are currently no improved BGN cultivars on the market. Farmers currently use the retained seeds of landraces, which are a mixture of pure lines with different quantitative and qualitative traits. However, the improvement of the BGN legume is challenged by its physiology and morphology, which are resistant to artificial hybridisation.

The current study is one of very few studies that have evaluated true breeding lines derived from genetic crosses from diverse parental lines, in order to assess the variations in the agro-morphological traits and cooking quality properties in BGN genotypes. The characterisation of germplasm is the first step towards helping plant breeders to identify lines with desirable traits for selection. Many studies have evaluated the nutritional composition of BGN seeds; however, the impact of traditional cooking on their nutritional composition is not fully understood. This study ascertains the feasibility of breeding fast-cooking BGNs with a high nutritional content. The results of this study will be useful in BGN breeding programmes, as the cooking-time will be added to other important agro-morphological traits in BGN production. This study is therefore foundation for any further genetic studies on the cooking time of the BGN.

1.4 Aims and Study Objectives

This study aims to identify BGN genotypes with desirable agro-morphological traits and cooking quality properties, in order to recommend lines that can be used for future plant breeding programmes.

1.4.1 Hypotheses

i) BGN genotypes show variations in their agronomic and morphological traits, as they are genetically different.
ii) BGN genotypes show variations in their cooking time, and their nutritional composition is negatively affected by cooking.

1.4.2 Specific objectives

The specific objectives of this study were:

i) to evaluate the genetic diversity among the Recombinant Inbred Lines (RILs) of the BGN, using agronomic and morphological traits for future plant breeding programmes; and

ii) to assess the cooking quality properties and nutritional composition of selected BGN RILs.

1.5 Definition of Terms

Agro-morphological trait: This is an observable characteristic of a plant that is a result of its genes and environmental factors (Chen, 2020).

Bambara groundnut: This is a highly-inbred, self-pollinating annual crop belonging to the Fabaceae family, and sub-family Faboidea, under Plantae (Bamshaiye et al., 2011).

Cooking quality: This refers to the cooking time, splitting during cooking, the texture and the sensory attributes (Mwangwela et al., 2006).

Recombinant inbred lines: These are a collection of strains that can be used to map the quantitative trait loci (Pollard, 2012).

1.6 Dissertation Structure

Chapter 1: Introduction, the Problem and its Setting

This chapter presents the introduction to this thesis; it highlights the background and importance of the study, the problem statement, its justification, its objectives and an overview of the research project.

Chapter 2: Literature Review

This chapter provides a detailed scoping review of the existing literature. It focuses on the mechanisms that are responsible for the development of the HTC traits, the strategies used to manage this problem and their impact on its nutritional composition.

Chapter 3: Assessment of the Genotypic Diversity of the Bambara Groundnut, based on its Agro-morphological Traits

The chapter clarifies the agronomic and phenotypic diversity of BGN, and the results are presented and discussed.

Chapter 4: Assessment of the Physical Traits, Cooking Quality and Nutritional Composition of Bambara Groundnut Genotypes

This chapter provides a detailed report on the physical characteristics, cooking properties and nutritional composition of BGNs used in this study.

Chapter 5: Summary, Implications and Recommendations

This chapter includes a general discussion of the findings of the experimental chapters, as well as the implications thereof. In addition, it provides the conclusions and recommendations for future research.

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CHAPTER 2
EVALUATION OF THE STRATEGIES USED IN THE MANAGEMENT OF THE HARD-TO-COOK (HTC) TRAIT IN LEGUMES: A SCOPING REVIEW

2.1 Abstract

BGN (*Vigna subterranean* (L.) Verdc.) is a nutritious and multiple stress-tolerant indigenous African legume; however, its potential to contribute to the region's food and nutrition security is constrained by limited research interests. One of the factors limiting the wider utilisation of BGN dry seeds is the 'HTC' phenomenon. The dry seed requires a prolonged boiling time, and therefore a high energy requirement, which limits the consumption of BGN. This challenge is not peculiar to BGN, but also to many legumes. Hence, a scoping review approach was used to explore the depth and breadth of knowledge that is available on 'the HTC' problem in pulses, to formulate strategies for developing BGNs with an enhanced cooking quality. A search on two electronic databases (Scopus® and the Web of Science®) identified 59 relevant articles. The findings showed that the storage conditions, which are characterised by high temperatures and humidity, the seed age or storage time, as well as the genetic make-up, are the primary causes of the development of the HTC trait in legumes. The most commonly reported method that is used to reduce the cooking time is the use of a salt solution. The genetic basis of the HTC phenomenon was also reported, which indicated the suitability of the trait to crop improvement. The Mattson bean cooker, texture analysing machines and finger pressing are used for measuring the traits that are correlated with HTC (hydration capacity, water absorption and stone seed percentage) and are the most common methods used to phenotype the cooking time and legume grain texture. A holistic approach should be used in the management of the HTC trait, as it will take into consideration the nutritional and health impact of the methodologies used to reduce the cooking time.

Keywords: Cooking quality, hard-to-cook, legumes, scoping review

2.2 Introduction

Over the past few decades, the growing world population has increased the pressure on the limited food resources, especially in regions where the effects of climate change are most severe (Teluguntla et al., 2015). Worldwide, only a few crops contribute to the bulk of human energy intake, since there is an over-reliance on wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays L.*) for subsistence. Recent evidence suggests that the productivity of most of these traditional crops is declining, due to the rising temperatures and their sensitivity to water stress. On the sub-Saharan African continent, climate change is affecting the productivity of staple cereals, which is causing major food and nutrition security concerns. In addition to developing crops that match the current and future climates, the research community must consider developing sustainable indigenous food crops (Thangjam, 2016).

Researchers have often focused on developing improved varieties of the world's major food crops such as wheat, rice, and maize (Bellon,2006), to tackle the global challenges, such as food and nutrition security, in the face of climate change. While this is understandable and necessary, thousands of indigenous and underutilised crops have been neglected. Most of underutilised crops have been identified as drought tolerant, resistant to pest and diseases and adapted to semi-arid environments (Mabhaudhu et al., 2017). The production of major crops might be inadequate to provide for the future population (Khan et al, 2017). Until recently, unlocking the potential value of these underutilised crops for addressing the effects of climate change and food and nutritional insecurity has not been the mainstream approach. However, some underutilised crops are now beginning to gain popularity because of their superior nutritional and dietary qualities and their ability to adapt to environmental stress.

BGN is one such indigenous and underutilised legume crop; it is protein-rich and adapts well to marginal land, which characterises the subsistence and smallholder farming systems of SSA (Chai et al., 2017). The crop is commonly cultivated in areas that are marginal for most crops, namely, those with a low fertility and acidic soils. It can also grow in areas with less than 500 mm of rainfall in the semi-arid tropics, to more than 2000 mm in the humid tropics. In many of these regions, the rainfall distribution is not uniform, and there can be prolonged periods of drought (4 to 7 weeks) during the crop cycle. BGN can boost the food and nutrition security because of its inherent tolerance to stressful environments and its ability to produce some yields in soils that are too poor for the cultivation of other leguminous species, such as the common bean (*Phaseolus vulgaris* L.) and groundnuts (*Arachis hypogea* L.) (Khan et al., 2021).

BGN crop can be considered a ‘super food’ because it has all the essential nutrients in their proper proportions. The legume has a considerable number of essential nutrients for the human diet; it provides proteins, complex carbohydrates, vitamins, mineral elements and dietary fibre, while it is low in cholesterol, fats and sodium (Broughton et al., 2003). Its complex carbohydrates and low oil content are of great importance for human health, especially for people with diabetes. BGN protein is rich in essential amino acids and methionine (Brough & Azam-Ali, 1992). In recent studies, the milk extracted from soaked BGN was reported to be the most preferred milk, due to its flavour and composition, when compared to the milk prepared from other legumes, such as cowpeas (*Vigna unguiculata*), pigeon peas (*Cajanus cajan*) and soybeans (*Glycine max*) (Brough et al., 1993). There has recently been an increased interest in the utilisation of nutraceuticals, which has positioned BGN as a potential source of bioactive components that can be exploited to improve health and nutrition (Okafor et al., 2022).

Despite its potential contribution to food and nutrition security, the crop remains classified as an ‘orphaned’ crop species (Aliyu et al., 2014). To date, there are no registered BGN varieties. Farmers still use landraces that were developed by means of mass selection over many generations. Some of these landraces carry important adaptive traits, but they are low yielding overall. The additional problem is that BGN (along with many other legumes) has the HTC phenomenon, which may provide some protection from storage pests, but it often requires expensive and hard-to-obtain fuel to rehydrate and cook (Adzawla et al., 2016; Mubaiwa et al., 2017: Gwala et al.,2019).

Seed hardness is a factor that causes an extended cooking time in legumes (Sandhu et al., 2018). The HTC trait is a severe problem in Africa, especially in communities that rely on firewood to provide energy for cooking and heating (Stanley, 1992). In SSA, the use of firewood for cooking is as high as 76% (Maes & Verbist, 2012), which leads to excessive deforestation. The prolonged cooking time also has implications for gender equality, since women will spend much of their time fetching firewood and cooking, at the expense of being involved in other productive activities. In addition, the prolonged cooking period of many legumes leads to the loss of nutrients (Addy et al., 2020), as well as structural changes, at a cellular grain level, which results in the loss of micronutrients, such as zinc and iron, which are important for mitigating malnutrition (Mukai, 2017). The hardness of the seed is also associated with a loss in the canning quality, in the nutritional quality and in uneven germination (Stanley, 1992).

The cooking time of legumes is influenced by environmental and storage conditions (Garruti & Bourne, 1985; Yousif et al., 2007), and it also controlled by specific genes like all the other heritable traits (Williams & Singh, 1987; Jacinto-Hernandez etal.,2003; Arns et al.,2018). Storage at high temperatures (30-40℃) and at a high humidity (< 75%) cause a physical alteration in the cell structure of grain legumes, which are associated with the HTC phenomenon and their poor cooking quality (Reyes-Moreno & Paredes-Lapez, 1993). Some studies propose that storage under adverse conditions results in the migration of tannins and polyphenols from the seed coat to the middle lamella, where they crosslink with pectins, causing the formation of insoluble pectates (Stanley., 1992 & Wainana et al.,2021). Lipid oxidation is also one of the primary causes of the HTC trait, due to membrane damage that occurs after storage under adverse conditions (Moscoso et al., 1984& Chigwedere et al.,2019).

The genetic variability of the cooking time is caused by multiple differences in the physical and chemical composition of seeds, which is expressed at various stages of the cooking time (Bassett et al., 2021). Some studies have confirmed that the cooking time is highly heritable and that it is controlled by a few genes (Jacinto-Hernandez et al., 2003; Cichy et al.,2019). The storage of grain legumes under low humidity- and low temperature-conditions can prevent the development of the HTC phenomenon. However, this is very difficult and not practical in most rural communities; hence, there is a need to explore the genetic factors that control the cooking-quality traits. The seed size, seed-coat colour and thickness, the size of the micropyle and hilum and the chemical composition of cotyledons can influence the cooking quality (Mkanda et al., 2007).

A short cooking time or ‘easy-to-cook’ traits are widely emerging as the consumer-preferred traits that are necessary for the variety to be accepted and adopted (Torga et al., 2011). Fairly recently, the HTC problem was resolved in some legume species; for example, in the common bean (Paredes-López et al., 1991; Gwala et al.,2019), cowpeas (Taiwo et al., 1997; Kayitesi et al., 2013; Obasi et al., 2014) and chickpeas (Ahmad et al., 1990). Some of the methods that have been adopted to reduce the cooking time in many legumes include cooking them in water or an alkaline solution, micronisation, pressure cooking and dehulling (Nakalema, 2015). This scoping review will help plant breeders to develop varieties with an improved cooking quality. It will also help them to understand the mechanisms that are responsible for the development of the HTC trait, to identify the strategies that are used to reduce the cooking time, as well as the methods that can be used to improve the cooking quality traits.

The variations in the cooking time of legume grain seeds will enable plant breeding for an improved cooking quality. The phenotypic characterisation of BGN cultivars is important for identifying lines that could be used as parents in plant breeding programmes, in order to develop varieties with short cooking times. Despite the supporting evidence in the literature on the inherent characteristics of the HTC trait, limited efforts have been made to investigate the potential of breeding crops with short cooking times, particularly among the indigenous orphaned crops. This scoping review aims to gather together the existing literature and identify the knowledge gaps, in order to advise policymakers, plant breeders, stakeholders in academia, and manufacturers on ways to improve and reduce the cooking time, and to increase the utilisation of this crop and other legumes that have HTC traits.

2.3 Materials and Methods

A systematic literature search was conducted on two electronic databases, www.scopus.com and www.sciencedirect.com; these were chosen due to their broad information coverage in agriculture sciences. A five-stage scoping review framework was conducted, as described by Arksey & O'Malley (2005). The framework outlines the stages involved in mapping the literature, namely: (i) the identification of the research question; (ii) the identification of the relevant studies; (iii) the study selection; (iv) the charting data; and (v) the collating, summarising, and reporting of results.

2.3.1 Research question

This scoping review aimed to provide a complete understanding of the HTC phenomenon in legume grains. This was achieved by identifying the different mechanisms that are responsible for the HTC trait in legumes and by highlighting the strategies that have been used to overcome it. As well as their impact on the nutritional and cooking quality of legumes, in order to identify the knowledge gaps in the literature and to provide recommendations for future research. The following research question and three sub-questions were used to achieve the objective of this review:

What are the strategies that have been used to manage the HTC trait in legumes?

Sub-question

i) What different strategies have been reported to have been used to manage the HTC trait in legumes?

ii) What are the effects of various strategies employed to manage HTC trait on the nutrient composition and quality of legumes?

2.3.2 The identification of relevant studies

Published literature from two electronic databases, namely Scopus® (www.scopus.com) and Science Direct® (www.sciencedirect.com), were used. The broad key terms 'hard to cook' and 'legumes' were used to identify articles without bias. The identified articles were imported to Endnote 20, and duplicates were removed. The screened articles were then exported to Distiller Sr for further screening. Articles were relevant if they discussed mainly legumes and the HTC phenomenon. Articles were included if they described the four attributes that are listed below:

i) studies that aimed to explain the mechanisms responsible for the HTC defect.
ii) studies that aimed to describe and assess the effectiveness of the different methods used to determine, or measure, seed hardness.
iii) studies that aimed to explain the strategies used to alleviate the HTC defects and to assess the nutritional impact of the methods used; and
iv) studies that assessed both the industrial and domestic suitability of the strategies used to reduce the hard-to-cook phenomenon.

2.3.3 Article screening

Title screening was conducted, and a screening question was used, namely: Is the title relevant to the study? If the answer was 'Yes', the article was included; and if it was 'No', the article was excluded. This was followed by the screening of the Abstract, and the screening question was used: Is the Abstract relevant to the study? If the answer was 'Yes', the article was included, if it was 'Maybe', the article was also included, and if the answer was 'No', the article was excluded. The last type of screening was related to full-text screening; only articles with the available full texts were used at this stage. A screening question was also used, namely: Is the full-text article relevant to the study? If the answer was 'Yes', it was included; if it was 'No', it was excluded.

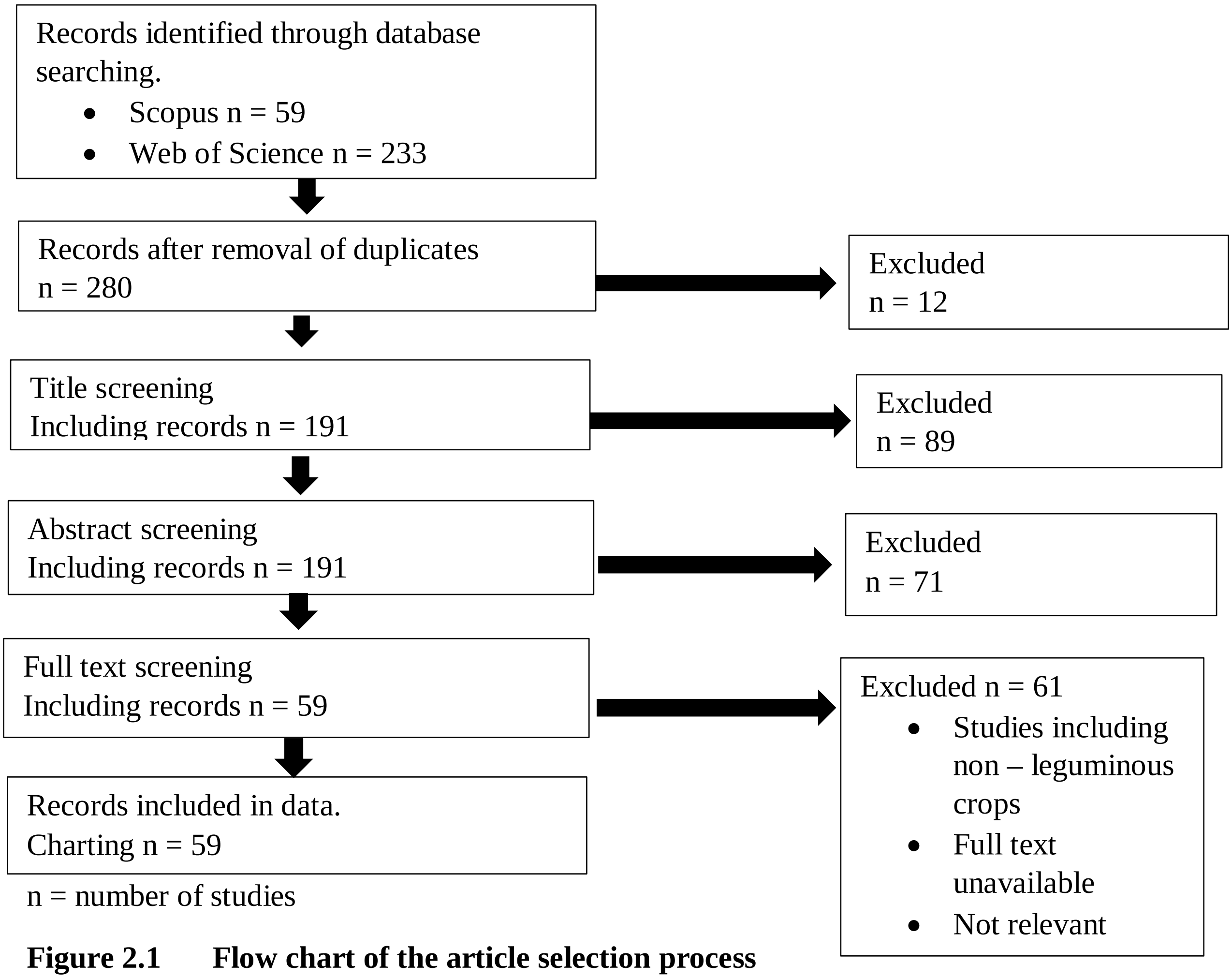
2.3.4 Data charting and analysis

A tabular data extraction form was created by using Microsoft Excel. Information on the name/s of the authors, the year of publication, the country of study, the main objective of the study, the cooking and texture analysing method, the legumes studied, the mechanisms responsible for the HTC trait and the strategies used in the management of this trait, their effects on the nutritional composition, as well as the conclusion, was extracted from each study. Pie charts and bar graphs were created by using Microsoft Excel.

2.4 Results

2.4.1 Summary of studies identified.

In this review, 292 articles were identified, and 59 articles were included in data charting. Figure 2.1 presents a flow chart of the article selection process.



n = number of studies

Figure 2.1 **Flow chart of the article selection process**

The selected articles were published between 1979-2022, but most of the articles (30) were published from 2010 to the present date (Figure 2.2).

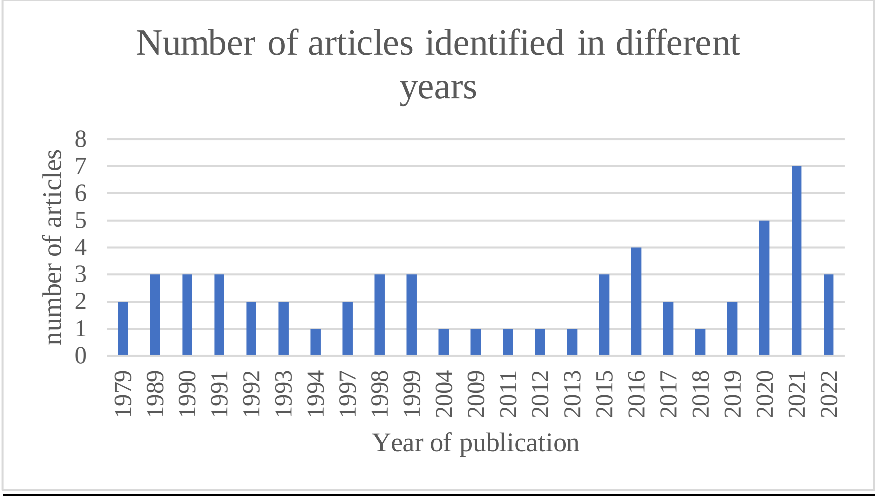


Figure 2.2 **Illustration of the time in which the articles used in this review were published**

2.4.2 Hard-to-cook legumes

In different studies, the common bean, and its varieties, including the carioca, pinto, kidney or black bean, were the most studied. Common bean is one of the major grain legumes consumed worldwide for its edible seeds and pods (Heuzé et al., 2015). Figure 2.2 illustrates the percentages of different legumes studied in the identified articles. Most legumes received little attention and this could be due to lack of market and seed (Kabambe et al., 2008).

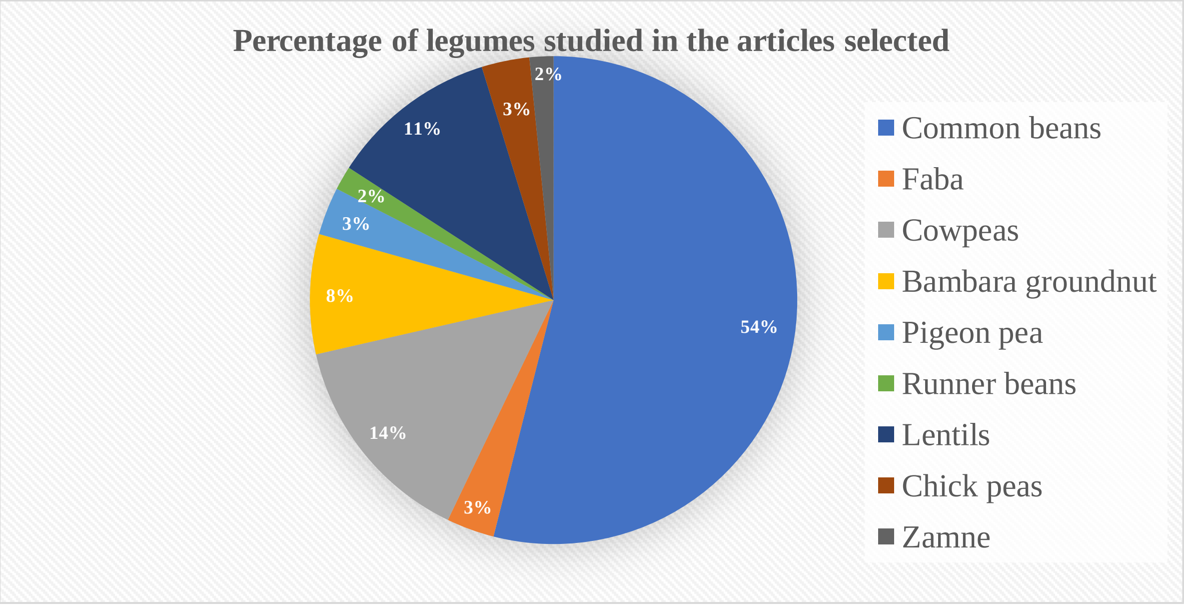


Figure 2.3 **Illustration summarising the legumes studied in the identified articles.**

2.4.3 Cooking methods

The main objective of only eight articles was to focus on describing the different cooking methods that measure seed hardness. The cooking time and hardness were the main traits used to measure HTC traits. Seven different cooking methods were identified from this review, with traditional cooking and the Mattson bean cooker being the most commonly-used methods, with an almost similar frequency, as illustrated in Figure 2.4.

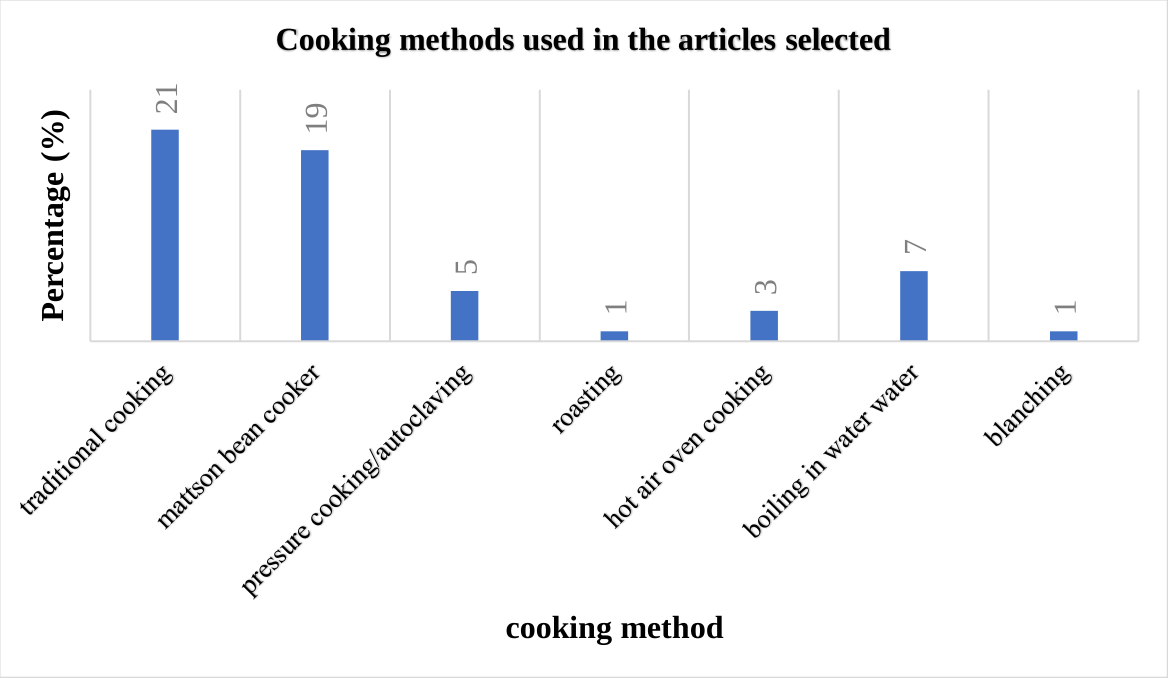


Figure 2.4 Cooking methods used in the identified studies

2.4.4 Mechanisms for the development of the hard-to-cook trait

Three main groups were identified as the major categories for classifying the mechanisms responsible for the development of the HTC trait, as illustrated in Figure 2.5. Fifty-four percent of the reviewed studies described the storage conditions as the leading cause of this trait, whilst 32% was attributed to the storage time and seed age, and the remaining 13% to their genetic make-up. The storage of legumes under adverse conditions, such as phytic acid reduction, pectin in solubilisation, divalent cation release and attachment to pectin, has a detrimental effect on their physicochemical properties (Pirhayati et al., 2011). Beans stored under elevated temperatures and relative humidity experience significant moisture loss during storage, prolonged hydration time and increased seed hardness when cooked (Yousif et al., 2002).

The longer cooking time in aged beans is related to pectin modification during ageing, which strengthens the middle lamella (Njoroge et al., 2016), as well as the presence of calcium cross-linked pectin (Chen et al., 2021). Although the genetic factors affecting seed hardness are not fully understood, a few studies confirmed the relatedness and heritability of the cooking time of legumes (Addy et al., 2020; Argel et al., 1999; Sandhu et al., 2018).

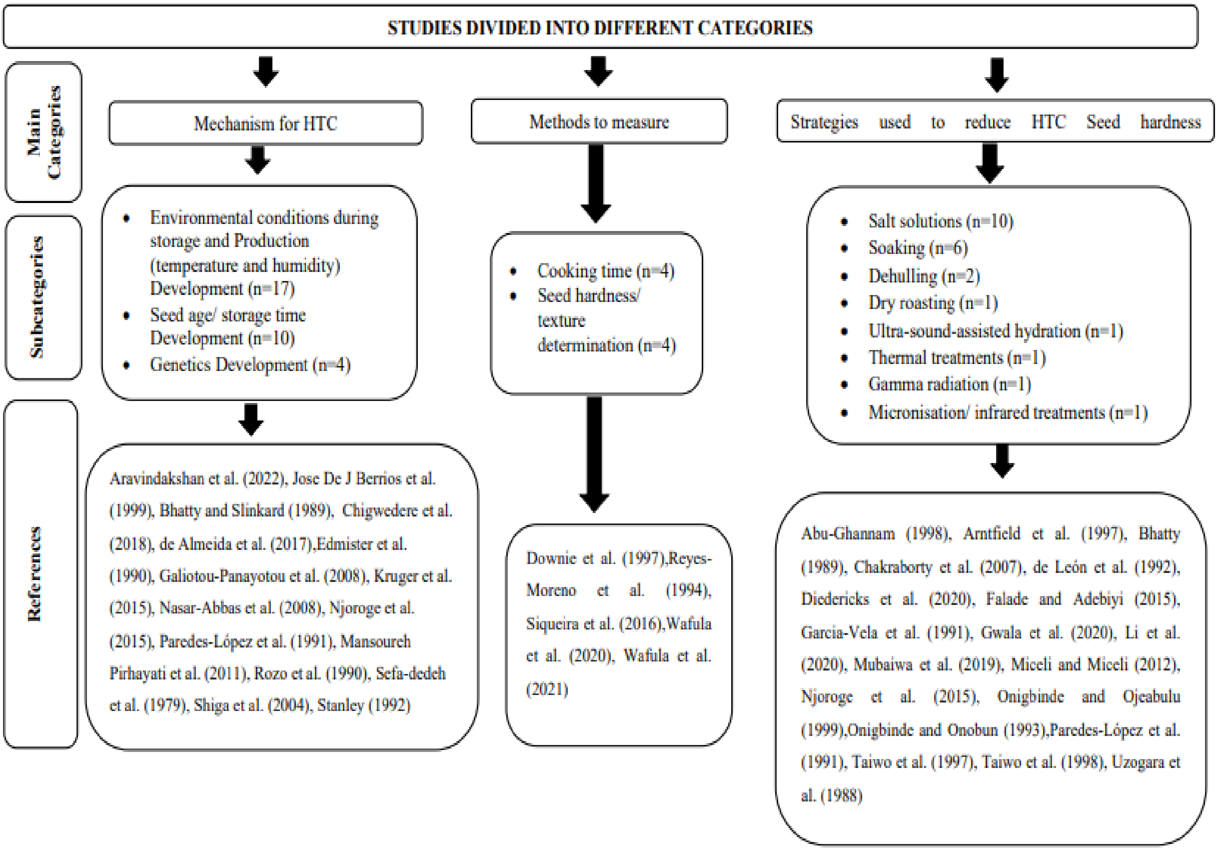


Figure 2.5: The studies used in this review divided into three main categories.

2.4.5 The management of hard-to-cook traits.

The most common strategy used to manage the HTC trait is the use of a salt solution (10 articles) followed by soaking them in water (six articles) (Figure 2.5). Roasting, ultrasound-assisted hydration, thermal treatments, gamma radiation and infrared treatments were the least investigated strategies in this review, each with only one article. The three major mechanisms identified as being responsible for legume softening are the structural, compositional, and physical changes. Table 2.1 summarises three major groupings of these articles, based on their objectives, which either focused on the mechanisms of development of the HTC traits, the methods used to measure seed hardness, or the strategies used to reduce the seed hardness or cooking time.

Table 2.1 Summary of the articles on the management of the HTC trait and the mechanisms involved

Author	Objective	Mechanism	Nutritional effect	Conclusion/s
Uzogara et al., (1988)	To evaluate the optimal conditions for cooking peas with kanwa and sodium bicarbonate and to assess the quality changes and mineral content of the cooked product.	Physical changes and compositional changes	Pressure cooking increased the total mineral (% ash) of beans; however, other minerals decreased due to leaching.	Atmospheric boiling in an alkaline solution decreased the cooking time, but increased the softness, leached solids and water absorption. The use of kanwa enhanced the cooking quality.
Bhatty (1989)	To determine the relationship between nutrient loss and the cooking of lentils treated under laboratory conditions.	Compositional change	Not investigated.	Phytic acid is the major determinant of shear force, and hence, the cooking quality of lentils.
Paredes-López et al., (1991)	To compare the effects of the cooking solution on the cooking quality of beans that have been hardened by the chemical method and storage under adverse conditions.	Structural changes	Not investigated.	Beans hardened by chemical and storage procedures may be softened by soaking in NaCl + $NaHCO_3$ or $NaHCO_3$ alone.
de León et al., (1992)	To evaluate the effects of using different ratios of monovalent to divalent ions on the cooking time, protein quality and sensory characteristics of common beans.	Physical and compositional changes	Alkaline solutions result in the racemization of amino acids to DL form and affect protein quality. Protein digestibility was not affected by using salt solutions.	Data indicate the feasibility of implementing this, at both an industrial and consumer level, to reduce cooking time.
Onigbinde & Onobun (1993)	To investigate the effect of the pH of the cooking water on the cooking property of cowpeas.	Structural changes	Not investigated.	HTC can be alleviated by using alkaline cooking water.
Reyes-Moreno & Paredes-Lapez (1993)	To investigate the mechanisms by which an aqueous sodium salt solution renders HTC beans easier to prepare.	Compositional changes	Not investigated.	Mechanisms for the action of salts depend upon protein solubilisation.

Table 2.1 Summary of articles on the management of HTC and the mechanisms involved (continued)

Author	Objective	Mechanism	Nutritional effect	Conclusion/s
Taiwo et al., (1997)	To study the water absorption characteristics of cowpeas when soaked at different temperatures for varying lengths of time.	Compositional changes	Not investigated.	Long cooking time can be reduced by soaking the beans at elevated temperatures before cooking.
Chakraborty et al., (2007)	To study the effects of chemicals, soaking and cooking time on the characteristics of instant pigeon peas.	Compositional changes	Not investigated.	Long cooking time can be reduced by soaking.
Chigwedere et al., (2018)	To determine polysaccharide changes of wonder beans occurring after soaking in demineralized water, Na2CO3, CaCl2 solution, followed by thermal treatment	Compositional changes	Not investigated.	Pre-treatment with Na2CO$_3$ resulted in shorter cooking time, whilst CaCl$_2$ solution hindered softening of beans.
Diedericks et al., (2020)	To gain insight into the structural changes of BGN seeds after various pre-treatments.	Structural changes	Soaking then roasting and roasting without soaking as pre-processing treatments reduced the protein content	Roasted seeds resulted in changes in the molecular composition of the resulting flours and protein isolates, whereas soaking had a minimal effect.
Drabo et al., (2020)	To map the variability in physical, chemical and cooking properties of Zamne obtained at local markets.	Physical changes	Traditional cooking with alkaline salt decreased the protein dispersibility index, carbohydrate digestibility and the leaching of water-soluble nutrients.	Traditional cooking of Zamne exhibited over-processing defects, including fat destruction and the extensive leaching of nutrients.
Li et al., (2020)	To explore the kinetic equation of water absorption during the soaking of black beans.	Physical changes	Not investigated.	Results can be used to predict the amount of water absorbed at different temperatures and changes in the proton population.
Vásquez et al., (2021)	To study ultrasound-assisted hydration of pigeon peas by using different concentrations of NaHCO3 in soaking water.	Structural and compositional changes	Not investigated.	Ultrasound is more efficient in improving the hydration of pigeon peas than cooking.

2.5 Discussion

This scoping review used standard review methods to identify, select and synthesise the findings of 59 studies that reported the HTC phenomenon in legumes. The review documented the literature by analysing the geographic scope, the type of legume, the cooking method, the mechanism for developing seed hardness and the strategies used to reduce the cooking time. The results highlighted published studies from between 1979 and 2022, with 50.8% of the studies being published in 2010. The global production of major legumes has increased in recent years, and this can be attributed to an increase in the demand for low-cost protein sources. The 2030 United Nations Sustainable Development Goals, which indicated that the transformation of the global food systems must lead the ending of hunger and malnutrition, also contributed to the increased research on legumes. The Leg value project, which was funded by the European Nations, also provided scientific support and policies that were directed at increasing legume production (Sepngang et al., 2020).

The increase in legume studies in recent years could also be due to the global shift towards plant-based food, as it is the best option, given its environmental benefits (Cusworth et al., 2021; Stagnari et al., 2017). Of the 59 experiments, 40.7% were conducted in Africa, 27.1% in North America, 10.2% in South America, 13.6% in Europe, 6.8% in Asia and 1.2% in Australia. Of the 59 articles on legumes, 54% were on were on the common bean, 14% were on cowpeas, 11% were on lentils, 8% were on BGN, 3% were on chickpeas, faba beans and pigeon peas each, and the lastly zamne and runner beans were 2% each.

Different methods have been used to manage the HTC trait; these strategies use three major mechanisms, namely, the compositional, physical and structural changes, as identified by (Mubaiwa et al., 2017). The cooking process of legumes involves two steps: the absorption of water to attain equilibrium and texture softening. These two stages are affected by different physicochemical changes. In this review, most of the literature investigated the use of a salt solution to manage the HTC trait; however, only 50% of the articles discussed the nutritional and health impacts of using different salt solutions. All the salt solutions positively impacted the cooking time, except for $CaCl_2$, which hindered seed softening. This was due to an increase in the concentration of calcium ions and a decrease in the phytate concentration, which resulted in the increased hardness of the seed (Galiotou-Panayotou et al., 2008). Although a salt solution reduces the cooking time, it has been reported to significantly reduce the protein quality and sensory properties of cowpeas (Onwuka & Okala, 2003). A study by Drabo et al. (2020) also reported over-processing defects, for example, a decrease in carbohydrate digestibility and protein dispersibility due to the use of a salt solution, which has also been reported to cause a reduction in lipids and sugars (Minka et al., 1999). One of the novel technologies that has recently been reported to reduce the cooking time of legumes is ultrasound (Ulloa et al., 2015; Vásquez et al., 2021; Yildirim et al., 2012). However, this process consumes a lot of energy and cannot be adopted for domestic use. Furthermore, roasting has been reported to significantly reduce the cooking time of BGNs, although it decreases the protein content (Diedericks et al., 2020).

Most of the strategies that have been used to manage HTC traits to date have been reported to cause a reduction in nutrients (Oghbaei, 2016), consume a lot of energy and reduce the sensory properties of legumes (Owusu, 2015), while some methods are impractical for domestic use. Therefore, the most sustainable way of managing HTC traits is to breed short-cooking varieties. Despite extensive literature explaining the problem of the HTC trait, little effort has been made to select cultivars with a relatively short cooking time and to use them in breeding programmes to develop cultivars with a short cooking time. Evidence from the articles shows that researchers, consumers, and processors have been focusing on reducing the cooking time during cooking and processing, rather than on breeding short cooking-time varieties because genetic variability of cooking time is less understood (Cichy, 2019).

There are no documented registered BGN varieties in SA. BGN is one of the most underutilised legumes, hence there has been limited improvement in plant breeding work. The use of molecular marker-assisted breeding techniques could also be adapted to identify the major genes responsible for the cooking quality. There are many landraces of BGN among local farmers in rural communities; therefore, it is necessary to first characterise these landraces and investigate the genetic basis of their cooking times. This will be achieved by assessing the genetic diversity of BGN through phenotyping for their cooking quality. Phenotyping is very important for the identification of desirable traits in breeding programmes, and thus it will be important for developing varieties with a short cooking time and retain a good proportion of nutrients as well as colour after cooking.

2.6 Limitations

This review has applied a systematic and rigorous search strategy that has retrieved articles to answer the research questions. However, articles that were not written in English may have been omitted. This study only used two databases and this could be another limitation to the outcome of this study. The findings of this review were restricted to published literature and prevented the analysis of relevant information that was rejected for publication by journals. Due to the characteristics of the scoping method, no quality evaluation of the studies was undertaken.

2.7 Conclusion

This scoping review acquired information that describes the mechanisms responsible for the development of the HTC trait and the strategies that are used in its management. The use of salt solutions, roasting, dehulling, thermal treatments, gamma radiation and micronisation are the most common methods to reduce the hardness of the legume seed. In the literature, little attention was given to the nutritional and health impacts of these methods. This study therefore paves the way for future research to evaluate the health and nutritional impacts of the different methods used to reduce the HTC trait. Future researchers should also focus on using molecular markers to identify the genes linked to the cooking time and they should use this information in breeding programmes.

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CHAPTER 3

ASSESSMENT OF GENETIC DIVERSITY AMONG BAMBARA GROUNDNUT RECOMBINANT INBRED LINES (VIGNA SUBTERRANEAN L. VERDC)

3.1 Abstract

BGN is a multi-stress-tolerant and nutritious legume that could make a significant contribution towards food and nutrition security across SSA. However, the crop continues to be classified among the neglected and orphaned crops. There are currently no improved BGN cultivars, and most farmers rely on the retained seed of landraces. This study reports the evaluation of BGN recombinant inbred lines that are derived from the successful hybridisation of four diverse parental lines, by using agro-morphological traits to select elite lines for further breeding. The experiment was laid out in a 90 x 5 augmented design with 21 lines, including parental lines as checks, at the Ukulinga Research Farm in SA. The agro-morphological traits were recorded at different growing stages, according to the International Plant Genetic Resources (IPGRI) descriptors. Data were subjected to an Analysis of Variance (ANOVA), Pearson correlations, a Principal Component Analysis (PCA) and a diversity index was also determined. Diverse qualitative traits were observed for the terminal leaf colour, the growth habits and the terminal leaf shape among the tested genotypes with different frequencies. Quantitative morphological traits, such as the days to flowering, the days to emergence, the internode length, as well as the plant height and petiole length, showed significant variations among the genotypes studied. A multivariate analysis of the quantitative morphological traits showed significant variations, which indicated that the first three PCs with eigenvalues ≥ 1 accounted for 80.6% of the total variance. The most important factor loadings for the first three PCs among the genotypes studied were the days to emergence, the days to flowering, the plant height, the petiole length, the grain yield per plant and the grain yield per plot. The days to emergence, days to 50% flowering, the plant height and petiole length were significantly and positively correlated to the grain yield per plant. The selection of these traits could be used to improve the grain yield of BGN genotypes. The significant differences among the tested genotypes for most of quantitative traits considered in this study, indicated that there is sufficient genotypic variation among BGN genotypes. Therefore, this study confirms the suitability of BGN for selection.

Keywords: Agro-morphologica traits, Bambara groundnut, genetic diversity, Recombinant Inbred Lines.

3.2 Introduction

BGN (*Vigna subterranean* L. Verdc) is a herbaceous, intermediate and annual crop that belongs to the Fabaceae family (Bamshaiye et al., 2011). Although the origin of BGNs remains controversial, some studies have traced it back to West and southern Africa (Hepper, 1963; Temegne et al., 2018). It is an underutilised crop that is mainly grown by women for family consumption (Ntundu et al., 2006). BGN is ranked the third-most important legume in Africa, after groundnuts (*Arachis hypogaea*) and cowpeas (*Vigna unguiculata*) (Adebowale et al., 2002). It is important as a plant-based protein source, especially for rural people who cannot afford animal protein Drechsel et al. (2001), as well as for animal fodder Bbebe (2019) and nutraceuticals Udeh et al. (2020), and it is a source of income for women in rural areas (Mubaiwa et al., 2017). The BGN is also drought-tolerant and produces better yields on poor soils, where most crops would fail. The world’s annual production of BGNs is estimated at 0.2 million tons Faostat (2009), with Nigeria accounting for approximately 50% of its production (Hillocks et al., 2012).

Despite its nutritional, agronomic and economic importance, BGN remains an underutilised crop, mainly due to an inadequate knowledge and documentation on its morphology and cultivation, as well as the lack of its genetic improvement (Tadele & Assefa, 2012). Morphological characterisation is considered to be the first step towards describing and classifying the genetic divergence of the existing germplasm (Cantini et al., 1999), while the characterisation of germplasm collections is traditionally based on their morphological and agronomic traits to aid selection. The use of agro-morphological markers is useful in the identification of unique gene combinations for desirable traits (Glaszmann et al., 2010). BGN has the potential to alleviate food and nutritional insecurity, especially in developing countries (Khan et al., 2021). However, there are no registered BGN varieties, and farmers currently use the retained seeds of landraces, which are a mixture of pure lines, with different combinations of quantitative and qualitative traits.

The complex anatomy of BGN reproductive system has been a major impediment to its improvement. The crop has minute florets that are resistant to emasculation and hand pollination. However, scientists at the University of Nottingham recently proved the suitability of BGN for artificial hybridisation. This novel discovery means that careful trait selection can now commence (Mayes et al., 2019), which could lead to the development of high-yielding BGN cultivars. The sustainable promotion and improvement of BGN requires the development and deployment of improved cultivars that are adapted to new climate conditions. Moreover, the development of new cultivars requires a clear understanding of the existing diversity, to inform breeding programmes and management strategies. In that regard, four BGN pure lines representing the available worldwide germplasm were recombined to develop two bi-parental populations, which were further advanced to F_3 and deployed to SA for further advancement and selection. Hence, this study is one of the very few experiments that have been conducted to evaluate the performance of true breeding BGN lines, in order to determine the agro-morphological diversity of the recombinant inbred lines and to identify the potential lines that can be used for future plant breeding programmes.

3.3 Materials and Methods

3.3.1 Study site

The study was conducted at the Ukulinga Research Farm in Pietermaritzburg, SA (30° 24`S, 29 °24`E) at 775 m above sea level. The area is characterised by hot summers and mild winters with a mean annual temperature of 18.4°C (Camp, 1997). The site receives an annual rainfall of about 680 mm. The soils are shallow clayey to clayey loam, with medium fertility (Mabhaudhi et al., 2013).

3.3.2 Planting material

The planting material consisted of 346 F5 RILs that were derived from four landraces from different locations in Africa and 21 check varieties, including the parents. The RILs were developed from the crosses of four parents S19, ANKPA 4, IITA686 and Lun T (Table 3.1) at the University of Nottingham in 2018, which were advanced to F3 and sent to the University of KwaZulu-Natal (UKZN) in 2019. The F3 families were advanced by using the single seed descent method from the F3 to the F5 generations.

Table 3.1 Bambara groundnut landraces used as parental lines for developing the RILs used in the study

Genotype	Geographical region	Location
ANKPA 4	7.24°N, 7.38°E	Ankpa, Nigeria
IITA686	6.10°S, 35.46°E	Tanzania
LUNT	8.29°N, 13.14°W	Lungi, Siera Leone
S19	22.33°S, 17.04°E	Namibia

3.3.3 Experimental design and management

The experiment was set up during the summer growing season, from December 2021 to May 2022, by using an augmented block design with 21 checks, including the parental landraces. The experiment was laid out in a 90 x 5 augmented design with 21 lines, including the parental lines as checks, at the Ukulinga Research Farm, Pietermaritzburg, SA. Both chemical and manual weed management were carried out at two-weekly intervals from the planting date. Pests and diseases were routinely managed by spraying them with karate Zeon (lambda-cyhalothrin) pesticide and copper oxychloride fungicides until the plants reached physiological maturity.

3.3.4 Data collection

Three qualitative and eight quantitative traits were observed for agro-morphological characterisation during the different stages of growth and development, based on BGN descriptors established by the International Plant Genetic Resources Institute (IPGRI, 2020) (Table 3.2).

Table 3.2 Quantitative traits observed and their brief description and codes

Quantitativ traits	Code	Type of measurement	Notation	Procedure
Days to emergency	DE	Counting	Days	The number of days from planting to the appearance of the first leaf on the soil surface.
Days to 50% flowering	DFF	Observation	Days	The number of days from emergency to when 50% of the plants have started flowering.
Internode length	IL	Tape measure	Cm	The average length of the 4th internode was randomly selected from the five longest stems at ten weeks.
Plant height	PH	Tape measure	Cm	The height of the main stalk, from the surface to the tip of the main panicle.
Petiole length	PL	Tape measure	Cm	The average length of three leaves at the 4th node of the five healthy plants at 10 weeks.
Canopy width	CW	Tape measure	Cm	The average widest length between two opposite points of five plants at 10 weeks.
Grain yield	GY	Weight	G	The weight of dried seeds at 12% moisture per plant
Grain yield per plot	GYP	Weight	G	The total weight of dried seeds at 12% moisture per plot

Qualitative morphological traits considered were grouped into growth habit, terminal leaf shape and terminal leaf colour traits and each group was represented by different character state (table, 3.3). Each genotype was scored for the most frequent character state.

Table 3.3 Qualitative traits observed and descriptor codes

Qualitative trait	Code	Measurement
Growth habit	GH	1-bunch type 2-semi-bunch 3-spreading type
Terminal leaf shape	TLS	1-round 2-oval 3-lanceolate 4-elliptic
Terminal leaf colour	TLC	1-green 2-red 3-purple

3.3.5 Data analysis

The frequency distribution and Shannon diversity index were calculated for the qualitative traits. The Shannon index (H’) was used to calculate the genetic diversity index of each trait, by using the following equation (Shannon, 1948):

$$\mathrm{H}' = -\sum_{i=1}^{n} Pi \ \mathrm{In}\ Pi \qquad (3.1)$$

Where (:)

Pi = the proportion (n/N)

N = number of species found in population N

ln = a natural logarithm

An Analysis of Variance for an augmented block design was carried out by using Rstudio software Version 4.1.3. Morphological quantitative traits that indicated a significant variation were subjected to a Principal Component Analysis Procedure (PRINCOMP) by using a correlation matrix to define the trait variation patterns using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2022). PCs with eigenvalues ≥ were selected to define the variation of the agronomic and morphological traits among the accessions.

3.4 Results

3.4.1 Frequency distribution for qualitative traits

The three morphological descriptors were used to determine plant variations among the studied BGN genotypes. Table 3.4 shows the qualitative traits, their variations, and their frequencies. The results showed that 58.5% of BGN genotypes that were studied have a bunch-type growth habit, 18.8% have semi-bunch-type habit and 22.6% have a spreading-type habit. Among BGN genotypes, 32.8% had round terminal leaflets, 40.1% had an oval shape, 3.3% had a lanceolate shape and 23.7% had an elliptic shape.

Table 3.4 Frequency of phenotypic traits studied among 451 genotypes

Trait	Variables	Frequency (%)
Growth habit	Bunch type	58.5
	Semi-bunch type	18.8
	Spreading type	22.6
Terminal leaflet shape	Round	32.8
	Oval	40.1
	Lanceolate	3.3
	Elliptic	23.7
Terminal leaflet color	Green	96.7
	Purple	3.3

3.4.2 Shannon-Weiner diversity index

Table 3.5 shows the diversity indices used in the study. The level of phenotypic variation of the three qualitative traits was estimated by using the Shannon diversity index (H’) (Table 3.5), which ranged from 0.2 to 1.19, with a mean of 0.78, indicating a high diversity. The terminal leaf shape showed the highest diversity, while the terminal leaf color showed the least variation.

Table 3.5 Shannon-Weiner diversity indices of the phenotypic traits of bambara groundnut genotypes

Trait	Shannon-Weiner index (H’)
Growth habit	0.96
Terminal leaflet shape	1.19
Terminal leaflet colour	0.201
Average diversity index	0.78

3.4.3 Analysis of Variance

The Analysis of Variance showed significant differences p<0.05 among the studied genotypes (checks + inbred lines) (Table 3.6). A significant block effect was observed in the days to emergence, the plant height, the grain yield per plant and the plot (1.5m single row); however, the block effect was not significant p>0.05 in the canopy width, the days to flowering, the internode length and the canopy width. The results showed a significant difference between the checks, as well as between the tests and checks, for most traits, except for the canopy width.

Table 3.6 Mean squares from the Analysis of Variance of augmented RCBD for eight parameters of bambara groundnut genotypes

Source of variation	d.f	CW	DE	DF	IL	PH	PL	GY	GYP
Treatment	364	113.12$^{n.s}$	12.31**	116.15**	9.05**	11.92**	26.76**	831.77$^{n.s}$	59.4$^{n.s}$
Check	20	109.77$^{n.s}$	44.73**	382.95**	5.99**	37.19**	88.72**	2701.8**	179.19**
Test vs check	1	17.29$^{n.s}$	86.46**	780.68**	4.59**	48.11**	383.01**	9773.59**	691.61**
Test	343	113.6$^{n.s}$	10.2**	98.65**	9.24**	10.35**	22.1**	696.66**	50.5$^{n.s}$
Block	4	26.44$^{n.s}$	3.00**	19.57$^{n.s}$	0.74$^{n.s}$	5.99*	6.26$^{n.s}$	7814**	528.73**
Residual	82	105.25	2.69	18.3	0.46	2.2	5.52	806.75	51.76

n.s indicates a non-significance p>0.05, ** indicates a significance p<0.05. **d.f**: Degrees of freedom, **GCV**: Genotypic Coefficient of Variation, **PCV**: Phenotypic Coefficient of Variation, **CW**: Canopy Width, **DE**: Days to Emergence, **DF**: Days to 50% Flowering, **IL**: Internode Length, **PH**: Plant Height, **PL**: Petiole Length.

3.4.4 Correlation analysis

The Pearson correlation results indicated an interrelationship among the studied traits. The highest significant correlation was observed between PL and DF (r = 0.878) (Table 3.7). The grain yield per plant exhibited a significant (p<0.05) positive association with DE (r = 0.216), DF (r = 0.259), PH (r = 0.246) and PL (r = 0.277). However, the grain yield per plant had a significant ($p<0.05$) negative association with the IL (r = 0.109). The results also indicated no significant correlation between the canopy width and other traits. Negative significant correlations were also observed between the IL and the DE (r = -0.233), the DF and IL (r = -0.246) and the IL and PH (r = -0.217).

Table 3.7 Pearson correlation coefficient (r) among eight quantitative traits of BGN genotypes

Traits	DE	DF	IL	PH	PL	CW	GY	GYP
DE								
DF	**0,867****							
IL	**-0,233****	**-0,246****						
PH	**0,749****	**0,862****	**-0,217****					
PL	**0,759****	**0,878****	**-0,214****	**0,854****				
CW	-0,007$^{n.s}$	-0,004$^{n.s}$	0,036$^{n.s}$	-0,040$^{n.s}$	-0,033$^{n.s}$			
GY	**0,216****	**0,259****	**-0,109****	**0,246****	**0,277****	0,003$^{n.s}$		
GYP	**0,210****	**0,254****	**-0,096****	**0,241****	**0,273****	-0,055$^{n.s}$	**0,870****	

DE: Days to Emergence, **DF**: Days to 50% Flowering, **IL**: Internode Length, **PH**: Plant Height, **PL**: Petiole Length, **CW**: Canopy Width**, GY**: Grain Yield per plot, **GYP**: Grain Yield per Plant, **significant $p<0.05$ and **n.s** not significant.

3.4.5 Principal Component Analysis (PCA)

The PCA for both the quantitative and qualitative traits are summarised in Table 3.8. The first three PCs with eigenvalues ≤ 1 accounted for 80.6% of the variability in the studied traits. PC1 accounted for 47.9% variation, and the days to emergence, the days to 50% flowering, the plant height and the petiole length showed the highest factor contributions in PC1. PC2 explained 20.2% of the total variation and was associated with the GYD and GYP traits. The canopy width was the main trait that contributed to PC3, and it was responsible for 12.6% of the total variance.

Table 3.8 Factor loadings, eigenvalues, variability, and cumulative variability for the five principal components

Trait	PC1	PC2	PC3	PC4	PC5
Days to emergence	0.868	-0.244	0.038	0.046	0.412
Days to 50% flowering	0.937	-0.226	0.045	0.061	0.054
Internode length	-0.326	0.041	0.278	0.903	0.019
Plant height	0.898	-0.217	0.007	0.097	-0.251
Petiole length	0.912	-0.185	0.019	0.100	-0.206
Canopy width	-0.040	-0.041	0.961	-0.268	-0.019
Grain yield per plot	0.477	0.839	0.061	-0.007	0.009
Grain yield per plant	0.472	0.844	0.001	0.026	0.013
Eigenvalue	3.8	1.6	1.0	0.9	0.3
Variability (%)	47.9	20.2	12.6	11.4	3.5
Cumulative (%)	47.9	68.0	80.6	92.0	95.5

3.5 Discussion

The plant growth habit is an important agronomic trait that determines the competitive ability of a legume crop (Wang et al., 2006). The vegetative growth of BGN was grouped into three categories, namely, the bunch, semi-bunch and spreading types. The growth habits showed considerable variations, with the bunch type being the most observed (58.5%), followed by the spreading type (22.6%). The least frequency was observed on the semi-bunch type. Similar to the results of this study, Gbaguidi et al. (2018) reported that a high proportion (40.4%) of BGN landraces were of the bunch type. This type is important to farmers, especially during harvesting, as it allows the easy pulling out of the plants, while the pods remain attached to the plant (Ntundu et al., 2006).

The leaf shape directly affects the most important physiological processes, such as photosynthesis, transpiration and thermoregulation (Nicotra et al., 2011). The highest terminal leaf shape was oval (40.1%), followed by the round (32.8%), elliptic (23.7%) and lanceolate (3.3%) leaf shapes. These results agree with those of Ntundu et al. (2006), who reported that the oval leaf shape was the dominating leaf shape in their study. The round and oval leaves are big and broad, and they increase the photosynthetic surface area (Chabot & Hicks, 1982). Genotypes with broad leaves are useful for fodder production (Unigwe et al., 2016), while those with narrow leaves (lanceolate) could also be used for drought tolerance breeding programmes (Ghafoor et al., 2001).

The ANOVA results showed that there were significant ($p<0.05$) differences among the tests and checks for all the analysed traits, except for CW (Table 3.6). The significant block effect was also observed in DE, GY and GYP (Table 3.6); however, CW showed no significant difference ($p>0.05$) among the treatments, checks and blocks. The ANOVA showed that there were significant ($p<0.05$) treatment effects for all the analysed traits, except for CW, GY and GYP (Table 3.6). The variations observed in the quantitative traits agree with those of (Valombola et al., 2020), who reported the presence of significant variations among twenty-five BGN accessions. Similarly, (Kambou et al., 2020) reported significant variations among ninety BGN accessions in twenty quantitative traits. The diversity of the different traits such as DE, DF, PH and GY in this study indicates the potential of improving BGN through selection and hybridisation.

A correlation analysis is a statistical tool that is used to measure the strength of the linear relationship between two continuous variables (Mukaka, 2012). Correlation coefficients have been widely used for selecting superior genotypes (Alam, 2014). The days to emergence, days to flowering, the plant height and petiole length showed a significant correlation with the yield, which suggests that selection based on these parameters may be useful for improving the yield. Similar results were also reported by (Zenabou et al., 2014; Gbaguidi et al., 2018), who observed some relationships between the morphological traits and the yield. The relationship between the yield per plant and other parameters is very useful in plant breeding, as it can be used as the basis for direct selection.

The PCA is one of the most common methods used for reducing data dimensionality, by using complex correlation structures among a large volume of numerical variables (Kim et al., 2009). This statistical analysis technique is commonly used to group phenotypic traits and to classify genotypes. The results of this study showed that the first two principal components accounted for 68% of the total variance, which is higher than the values (51.35%) reported by (Gonné et al., 2013), who assessed twenty BGN landraces by using quantitative morphological traits. The days to emergence, days to flowering and plant height contributed more to the variation, which implies that they can be used for selecting plants with a better performance. The results also indicated that these morphological traits were used during the selection programme.

3.6 Conclusion

There is a need to improve the utilisation of under-utilised crops to alleviate food shortages and malnutrition, especially in developing countries. This study aimed to evaluate the genetic variability of BGN RILs as a preliminary reference for future plant breeding programmes. The genotypes that were studied showed significant differences, which suggests the potential heritability of traits from parental germplasm for the improvement of BGN. The correlation analysis indicated that the days to emergence, the days to 50% flowering, the plant height and petiole length had a significantly positive effect on the yield; hence, the selection of those traits will improve the yield. Furthermore, it is important to undertake more research on the molecular characterisation of BGN genotypes.

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CHAPTER 4

ASSESSING THE PHYSICAL TRAITS, COOKING QUALITY AND NUTRITIONAL DIVERSITY OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEAN* L. VERDC) GENOTYPES

4.1 Abstract

BGN is a nutrient-balanced legume that has the potential to alleviate food and nutrient deficiencies. However, its utilisation is limited by its HTC traits. The objective of this study was to examine the physical traits, cooking quality (cooking time and texture) and nutritional composition of BGN. One-hundred-and-fifty-six genotypes were assessed for their physical and cooking quality traits, and twenty were selected for a nutritional analysis. Before cooking, BGNs were evaluated for their seed colour, weight, volume, density, hydration capacity, swelling capacity, pH, electrical conductivity and nutritional composition. The traditional cooking method was conducted, and the cooking time was determined by using the finger-pressing method. The texture and nutritional composition of the seeds were evaluated after cooking. Different genotypes showed significant variations in their cooking quality traits and proximate and mineral content composition. The seed size of BGN ranged from 0.23-0.86 g, the seed volume from 0.19-0.68 ml, the hydration capacity from 0.01-0.45, the swelling capacity from 0.02-0.6, the cooking time from 40-147 minutes and the texture from 5.4-33.1 N. Their hydration properties, swelling properties, seed size, texture, degree of lightness, pH and electrical conductivity were significantly correlated to the cooking time. The total mineral content (ash), fat and moisture showed significant variations ($p<0.001$), while the NDF and protein content showed a significant difference between raw and cooked samples ($p<0.05$). Cooking significantly increased the protein, fat, NDF, zinc, sodium and copper content; however, the process also significantly reduced the iron, moisture and total mineral (ash) content. BGN lines, S19/Ankpa4-100, S19/Ankpa4-92, IITA686/LunT-403, and S19/Ankpa4-234, could the used as sources of the reduced cooking time genes. The results could contribute to the genetic improvement of BGN. Plant breeders and dietitians/nutritionists should work together to develop genotypes that have a high nutritional content, that are fast-cooking and that have a desirable texture.

Keywords: Bambara groundnut, cooking quality, genotypes, nutritional composition, physical trait

4.2 Introduction

BGN is a sustainable, cheap source of complex carbohydrates, plant-based protein, unsaturated fatty acids and essential minerals (magnesium, iron, zinc and potassium), especially for people living in the arid and semi-arid regions of the world (Tan et al., 2020). The grain legume food crop is commonly grown by women, mainly to feed their families (Ntundu et al., 2006). Its seeds contain approximately 64.0% carbohydrates, 23.6% protein, 6.5% fat and 5.5% fibre (Halimi et al., 2019), and they are reported to be rich in micronutrients, such as iron (4.9-40 mg/100 g), calcium (95.5-99 mg/100 g), potassium (11.44-19.35 mg/100 g) and sodium (2.9-12 mg/100 g) (Tan et al., 2020). In addition to their nutritional value, BGNs have been reported to contain polyphenolic compounds, which indicates their potential for use as nutraceuticals (Nyau et al., 2015; Oluwole et al., 2021). BGN has been widely used as a traditional medicine in Africa to treat cancerous growths and inflammatory disorders (Na et al., 2004).

Food insecurity and micronutrient deficiencies are reported to be highly prevalent in SSA (WHO, 2021). Although SA is food secure at a national level, food insecurity remains a problem at a household level, with approximately 23.6% of South Africans being affected by moderate-to-severe food insecurity (Statistics SA, 2019). Micronutrient deficiencies are prevalent worldwide; they affect more than two billion people, and approximately nine million people die of hunger each year (WHO, 2019). Pregnant and lactating women, as well as young children, are particularly vulnerable to the impact of micronutrient deficiencies, due to the increased demands for reproduction and growth (Christian & Smith, 2018). The most common micronutrient deficiencies among women and children are iron, vitamin A, iodine, folate, and zinc (Bailey et al, 2015). Micronutrient deficiencies can affect development, can cause growth retardation, cause mortality and result in several other disease conditions, thereby affecting the human potential such as cognitive function, energy levels and mental wellbeing (Bain et al, 2013 & Black et al., 2013) more than 25% of all people worldwide (Wirth et al., 2017).

Despite the fact that BGN is important as a nutritionally-rich legume crop in Africa, it remains an underutilised crop, due to its poor cooking quality and HTC traits (Diedericks et al., 2020). The cooking quality is considered to be the most important quality parameter in grain legumes, with the cooking time, cooking loss, water absorption index, swelling index and texture being essential (Ficco et al., 2016). In order to increase the consumption of BGN and other grain legumes in developing countries, breeding programmes should aim at producing varieties that have better cooking and nutritional qualities than the existing landraces; however, very few efforts have been made to accomplish this (Iqbal et al., 2006).

Cooking is a process in which heat is applied to a food item and, in the case of grain legumes, this process makes them more tender and palatable (Carmody & Wrangham, 2009) and improves their nutritional quality (Wrangham & Conklin-Brittain, 2003). Most grain legumes are soaked before cooking to ensure uniformity during the expansion of the seed coat and cooking (Hoff & Nelson, 1965). This process influences the texture (Sefa-dedeh et al., 1979) and reduces the cooking time (Scanlon et al., 1998 & Naviglio et al ., 2013). The HTC phenomenon is the biggest constraint in the use of BGNs and other grain legumes, as it increases the cooking time. Due to the high energy costs associated with a prolonged cooking time, consumers in most developing countries now prefer short-cooking varieties (Atilola, 2018). The cooking quality of legume seeds has recently become very popular among consumers, manufacturers and researchers, because of its implications for nutrition and energy consumption; it is a complex trait that is related to the cooking time, texture and taste of the cooked product (Graham et al., 1999).

The cooking time is required to reach a cooked texture that is acceptable for eating; however, this depends on the seed's genetic factors Cichy et al. (2019), its physical properties (Ahmad et al., 1990), chemical composition (Sánchez-Arteaga et al., 2015), as well as the environmental conditions and storage time (Jackson & Varriano-Marston, 1981). Water absorption is an indirect criterion that is used to determine the cooking quality of most grain legumes (Sofi et al., 2014). The hydration capacity of the seed has also been reported to be correlated to the HTC phenomenon (McWatters et al., 1987; Singh et al., 2004), while some studies have reported a correlation between the morphological traits, such as the grain thickness, flatness and cooking time (Santos et al., 2016). A texture profile analysis is a useful method that can be used to evaluate the texture of grain legumes, while an instrumental texture analysis has gained prominence for assessing the hardness of grain legumes (Saha et al., 2009) because of its potential for high-throughput phenotyping.

The cooking time is a heritable trait that differs among the genotypes (Kaur et al., 2005; Williams & Singh, 1987); therefore, the characterisation of the cooking time is critical in breeding, in order to improve the cooking time and nutritional quality. The cooking process of legumes is crucial as it improves the availability of nutrients, although prolonged a cooking time has been reported to decrease nutrient retention (Wainaina et al., 2021). The cooking-quality traits of most legume crops are not often considered in most crop-improvement objectives. Although literature is available on breeding for a short cooking time in BGN, there is a lack of data on these experimental investigations and their implications for the management of the HTC trait. Short cooking varieties will help to increase the utilisation of HTC legumes, they will reduce energy consumption and help to alleviate food and nutrition insecurity, especially for people living in rural communities (Mubaiwa, 2018). Furthermore, plant breeders and dietitians/nutritionists must work together to develop nutritionally superior BGN varieties that have an appropriate cooking time, which consumers prefer. Hence this study aimed to identify the short cooking properties and the effect of soaking and cooking on selected nutrients in selected BGN recombinant inbred lines.

4.3 Materials and Methods

4.3.1 Bambara groundnut seed samples

One-hundred-and-thirty-five BGN F6 lines, were taken from the trial conducted in chapter three for this part of the study. BGN seeds were manually harvested and sun-dried. The seeds were stored for one month after harvesting at room temperature, before conducting the experiments. Figure 4.1 presents a summary of all the experimental procedures and data collected in this part of the study.

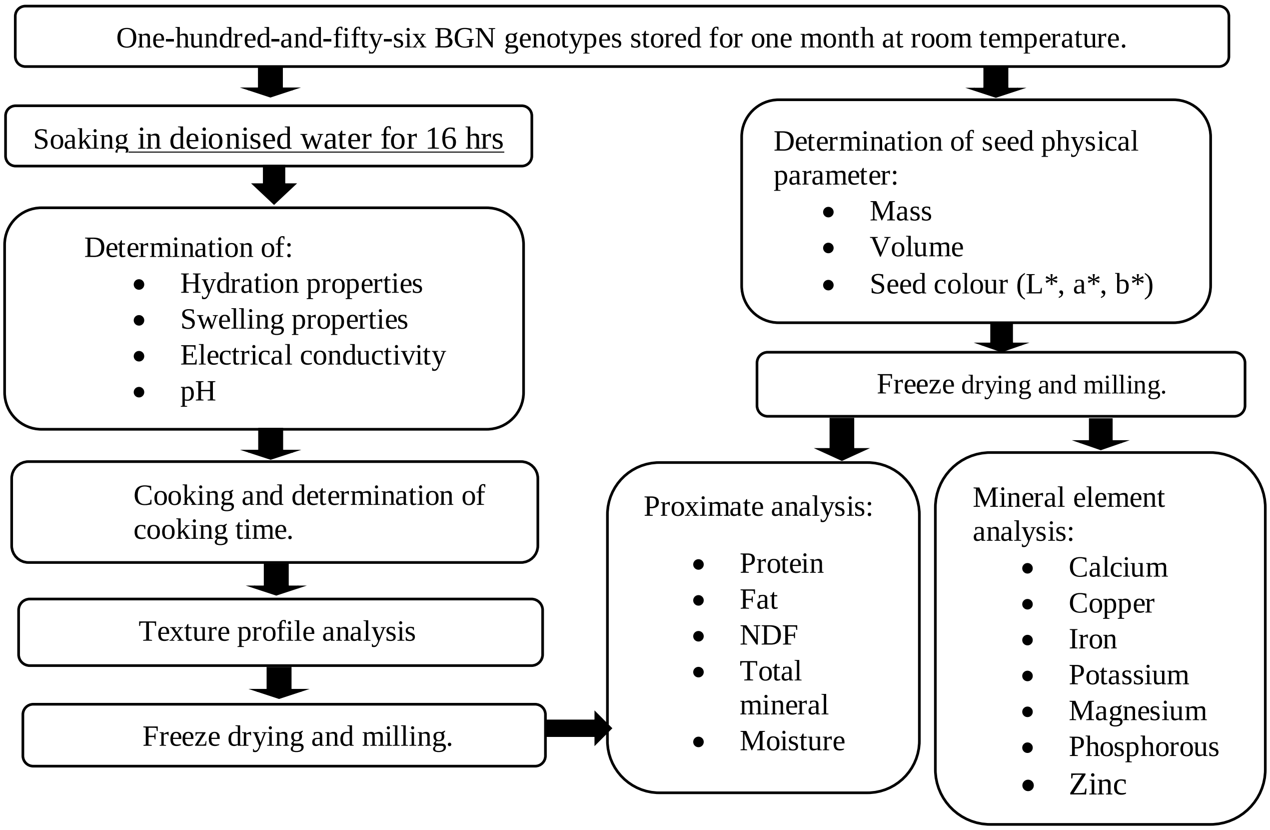

Figure 4.1 **Flow chart showing experimental methodology from seed storage to nutritional analysis**

4.3.2 Grain quality traits

BGN seeds were cleaned, and 50 disease-free, undamaged seeds were taken per genotype. The seed traits investigated in this study were the seed size, the volume, the density, the hydration properties, the swelling properties, the electrical conductivity, and the pH, and these were determined by using standard methods.

Seed size

The seed size of the samples was measured by using a calibrated digital electric scale Model PCE-BSH 600 and they were recorded as the mean weight of 50 seeds per genotype (Martín-Cabrejas et al., 1997).

Seed volume

The seed volume was determined by transferring 50 seeds to a 50 ml measuring cylinder with 25 ml of deionized water at room temperature. The seed volume (ml/seed) was calculated using equation 4.1 below (:)

$(total\ volume - 25)/50$ (Huma et al., 2008). (4.1)

Seed density

The above calculated seed weight and volume were used to determine the seed density, which was recorded as g/ml (Asoegwu et al., 2006).

Hydration capacity and index

The 50 seeds per genotype and 100 ml of deionized water were added to an Erlenmeyer flask and left for 16 hours at room temperature. The seeds were drained, the excess water was removed with absorbent paper and the swollen seeds were weighed on a calibrated digital electric scale Model PCE-BSH 600. The seed weight and volume calculated above were also used in this formula as the seed weight and volume before soaking, respectively (Williams et al., 1983). The hydration capacity was calculated as the difference between the weight before and after soaking, divided by 50. The calculated hydration capacity was divided by the seed weight before soaking, to determine the hydration index.

Swelling capacity and index

The soaked seeds were placed in a 100 ml measuring cylinder; 50 ml of deionized water was added, and the volume was recorded as the volume after soaking. The changes in volume were used to determine the extent of the swelling (Williams et al., 1983). The calculated swelling index was divided by the seed volume used before soaking, to determine the swelling index.

Leached electrolytes and pH

BGN seed samples were soaked in 100 ml of distilled water at room temperature for 16 hours. The electrolytes that were leached were quantified by assessing their conductivity by using a digital conductivity meter (Berrios et al., 1999). Both the pH and electrical conductivity of the soak water were determined by the Orion Star A215 pH/ conductivity Benchtop meter.

Seed coat colour

A Hunter Laboratory colorimeter (Hunter Associates Laboratory Inc, Reston, VA) was used to measure the seed coat colour of raw BGN seeds. The spectrophotometer was calibrated by a white tile. The seed colour was measured as L*, a* and b*, an international standard adopted by Commission Internationale d'Eldarage (CIE) in 1976. The seeds were placed in a glass cup and covered with a metal cup, and the colour measurement was taken. The sample was shaken to reduce any sampling errors due to seed colour variability, and the process was carried out in triplicate.

4.3.3 Preparation of the bambara groundnut samples

Each BGN seed sample was measured separately by using a 25 ml glass cup, and the quantity of BGN seeds depended on the seed size. BGN seeds were placed in a pot containing 200 ml of deionized water and then boiled on a Defy Thermofan Stove (Model 731 MF) at a heat setting of 5. The visual determination of the gelatinisation and the finger-pressing methods were used to determine the cooking level. The finger pressing method is one of the most common method used to determine cooking time. It is also simple, cheap, time-saving and does not require additional equipment. The cooked BGN seeds were pressed between the thumb and the forefinger, and the seed was recorded as cooked when the cotyledons disintegrated upon pressing (Kinyanjui et al., 2015). The samples were removed from the pot without interrupting the boiling, and the degree of cooking was tested at five-minute intervals. This procedure was repeated until all BGN samples were classified as being cooked. Seeds were considered to be cooked when five consecutive tested seeds were cooked, and the total cooking time was recorded at this time.

4.3.4 Texture analysis of the cooked grains

Once the samples were cooked, the texture of BGN was determined by using the Texture Analyser TA-TX2 (Stable Microsystem, Surrey, England). A return-to-start method was used for measuring the force under compression by using a Warner-Bratzler shear cell. A 2 mm probe was used to record the maximum peak force. The whole BGN seeds were axially compressed to 75% of their original height. The force-time curves were recorded at a speed of 2 mm/s, and the results were expressed in Newton (N).

4.3.5 Nutritional analysis

Ten samples with the shortest and 10 samples with the longest cooking times, were selected for nutritional analysis. The raw and cooked samples were freeze-dried and ground to a fine powder by using a coffee grinder, Model CBM4-B5. Five grams of fine powder per sample were taken to the Analytic Services Department at the Cedara College of Agriculture, SA, for a nutritional analysis (the moisture, fat, fibre, crude protein and total mineral content (ash). The nutritional analysis was conducted in duplicate by using the appropriate Association Analytical Chemists methods (AOAC, 1990).

Protein

The protein content of BGN samples was measured by using a LECO Truspec Nitrogen Analyser (Leco, Corporation, St Joseph, Michigan, USA) and the AOAC method 990.03 (AOAC, 1990). The samples were placed into a combustion chamber at 950℃, and measurements were taken in duplicate. The sample was combusted in an oxygen-rich atmosphere, and helium gas served as a carrier gas in the furnace. The protein content was calculated by using equation 4.2 below:

$$\%\ \text{Crude protein} = \%\ \text{N} \times 6.25 \qquad (4.2)$$

Fat

The fat content was determined according to the AOAC official method (AOAC, 1990), by using the Soxhlet extraction method with a Buchi 810 Soxhlet Fat extractor (Buchi, Flawil, Switzerland). Petroleum ether was used to extract the fat from the sample and to measure the weight of the recovered fat.

The percentage of crude fat was determined by using equation 4.3 below:

$$\% \text{ crude fat} = \frac{\text{Beaker+fat−Beaker} \times 100}{\text{sample mass}} \qquad (4.3)$$

Neutral detergent fibre (fibre)

BGN samples were dried, and the fat content was determined. All samples with a fat content of above 10% were then defatted before conducting this test. The enzyme amylase and neutral detergent reagent were added to the samples and boiled for one hour. The boiled samples were filtered using a low vacuum and dried overnight at 105℃. The samples were ashed for three hours at 550℃, and their weight was recorded before and after ashing. The NDF was calculated using equation 4.4 below:

$$\% \text{ NDF} = \frac{\text{(crucible+dry residue)−(crucible+ash)} \times 100}{\text{sample mass}} \qquad (4.4)$$

Total mineral content (ash)

The total mineral content was determined as ash, according to the AOAC method (AOAC, 1990). The samples were heated in a furnace at 550℃ to remove the organic matter. The percentage of ash was calculated using equation 4.5 below:

$$\% \text{ ash} = \frac{\text{(mass of sample+crucible after ashing)−(mass of pre−dried crucible)} \times 100}{\text{(mass of sample+crucible)−(mas of pre−dried crucible)}} \qquad (4.5)$$

Moisture content

The moisture content was determined by using the AOAC method (AOAC, 1990). The samples were oven-dried for 72 hours at 95℃, and the weight loss sample was regarded as having a moisture content. Moisture content was calculated by using the equation 4.6 below:

$$\% \text{ moisture} = \frac{\text{(mass of sample+dish)−(mass of sample+dish after drying)}}{\text{(mass of sample+dish)−(mass of dish without lid}} \times 100 \qquad (4.6)$$

Mineral elements

The mineral elements were determined according to the AOAC official method by using Atomic Absorption Spectrometry (AAS), which is equipped with a flame and a graphite surface. BGN samples were oven-dried at 105℃ for two hours and ashed in a furnace at 550℃ for four hours.

4.4 Data Analysis

The data were entered into a Microsoft Excel spreadsheet and transferred to the statistical package Genstat 20th Edition. A paired t-test was performed, using the Genstat 20th Edition to compare the means of proximate and mineral element composition of the raw and cooked BGN samples, and values $p<0.05$ were statistically significant. The Pearson correlation and a PCA were carried out by using XLSTATS ® to determine the relationships among the cooking-quality traits.

4.5 Results

4.5.1 Physical variability of the cooking-quality parameters

The results for the distribution of the cooking quality parameters among the 156 BGN RILs are presented in Table 4.1. The seed weight ranged from 0.25 to 0.83 g/seed, with S19/Ankpa4-112-96 having the smallest seed, and Uniswa having the largest seed. The seed volume and density ranged from 0.23 to 0.67 ml and 0.01 to 1.85 g/ml, respectively. The hydration capacity ranged from 0.02 to 0.42, while the hydration index ranged from 0.02 to 0.89. The swelling capacity and swelling index ranged from 0.02 to 0.47 and 0.03 to 1.60, respectively. Uniswa had the lowest swelling capacity and the largest seeds, as indicated above, whilst IITA686/LunT-403-314 had the highest swelling capacity.

Table 4.1 Descriptive statistics for the cooking quality traits of 156 bambara groundnut genotypes

Parameters	Minimum	Maximum	Mean	Std. deviation
Seed size	0.250	0.830	0.511	0.102
Seed volume	0.230	0.670	0.407	0.084
Density	0.010	1.850	1.257	0.169
Hydration capacity	0.010	0.420	0.100	0.086
Hydration index	0.020	0.890	0.210	0.194
Swelling capacity	0.020	0.470	0.104	0.087
Swelling index	0.030	1.600	0.281	0.263
Cooking time (min)	40.000	147.000	88.410	18.581
EC	4.460	9.870	7.424	1.126
Ph	5.330	8.880	7.042	0.719
L*	13.920	58.450	29.534	13.244
a*	0.560	22.920	8.717	6.276
b*	-0.040	33.940	11.715	11.575
Texture	5.400	32.700	18.504	5.566

.L* - degree of lightness, a* - degree of red to green, b* - degree of blue-yellow

The electrical conductivity ranged from 4.46 to 9.87 Kohm/cm, whilst the pH ranged from 5.33 to 8.88, and these two parameters were negatively correlated (Table 4.2). S19/ankpa4-100-87 had the lowest EC and the highest pH, while S19/Ankpa4-106-92 had the lowest pH and the highest EC. The hardness ranged from 5.4 to 32.7 N, and the cooking time ranged from 40 to 147 minutes. S19/Ankpa 4-106-92 had the longest cooking time, while S19/ankpa4-106-92 had the shortest cooking time. The lightness (L) values ranged from 13.9 to 58.4, and IITA686/LunT-403-314 showed the lowest value, while IITA686/LunT-403-314 showed the highest value. The degree of greenness to redness (a) and blueness to yellowness (b) ranged from 0.56 to 22.9 and -0.04 to 33.9, respectively.

4.5.2 Correlation Analysis

The Pearson correlation coefficient of fourteen cooking quality parameters is shown in Table 4.2. The results indicated that the seed size had a significantly strong positive correlation with seed volume (r = 0.88) and a weak negative correlation with the hydration index (r = -0.35). The hydration index had a significantly strong correlation with both of the swelling properties, with coefficients of r = 0.81 and r = 0.80 for the swelling index and swelling capacity, respectively. However, the hydration index was negatively correlated with the cooking time (r = 0.448) and texture (r = 0.445). The cooking time showed a significant positive correlation with the electrical conductivity (r = 0.94) and a negative correlation with the pH (r = -0.96). The degree of blue to yellow (b) was positively correlated to L (r= 0.97) and a (r= 0.756).

Table 4.2 Correlation coefficients among the cooking traits of 156 bambara groundnut genotypes

	SS	SV	D	HC	HI	SC	SI	CT	EC	pH	L.	a.	b.	Tex
SS														
SV	0.8825*													
D	0.2374$^{n.s}$	-0.1622*												
HC	-0.1077$^{n.s}$	-0.1327*	0.0522*											
HI	-0.3503*	-0.3394*	-0.0208*	0.9414*										
SC	-0.1046$^{n.s}$	-0.2315$^{n.s}$	0.2291$^{n.s}$	0.8318*	0.8027*									
SI	-0.2836*	-0.4156*	0.236*	0.7623*	0.8198*	0.9409*								
CT	0.1959*	0.2632$^{n.s}$	-0.1108$^{n.s}$	-0.4225*	-0.448*	-0.5463*	-0.5495*							
EC	0.208*	0.2616*	-0.0881*	-0.4422*	-0.4727$^{n.s}$	-0.5627*	-0.5611*	0.9484*						
pH	-0.1752*	-0.2586*	0.1258*	0.3566*	0.3828*	0.496*	0.4964*	-0.962*	-0.904*					
L.	-0.2354*	-0.2032*	-0.0314$^{n.s}$	0.3447*	0.3736**	0.3674*	0.3896*	-0.1604*	-0.1467*	0.1269$^{n.s}$				
a.	-0.0527$^{n.s}$	-0.0531$^{n.s}$	-0.0501$^{n.s}$	0.1086$^{n.s}$	0.1055$^{n.s}$	0.1243$^{n.s}$	0.1442$^{n.s}$	-0.0348$^{n.s}$	-0.0219$^{n.s}$	0.0442$^{n.s}$	0.6629			
b.	-0.2102*	-0.1836$^{n.s}$	-0.034$^{n.s}$	0.2962*	0.3242*	0.3171*	0.3353*	-0.1332$^{n.s}$	-0.1195$^{n.s}$	0.1045$^{n.s}$	0.973*	0.7567		
Tex	0.221*	0.2776*	-0.0912$^{n.s}$	-0.4237*	-0.4599*	-0.5621*	-0.569*	0.9627*	0.923*	-0.9184*	-0.1911	-0.0701	-0.1703	

SS: Seed Size, **SV**: Seed Volume, **HC**: Hydration Capacity, **HI**: Hydration Index, **SC**: Swelling Capacity, **SI**: Swelling Index, **CT**: Cooking Time, **EC**: Electrical Conductivity, **L**: degree of Lightness, **a**: degree of green-red, **b**: degree of blue-yellow, **Tex**: Texture, ***** and **n.s** indicates the significance at $p<0.05$ and not significant respectively.

4.5.3 Principal Component Analysis

The results of the PCA are shown in Table 4.3. The first four main components explain 85.9% of the total variations in the cooking quality parameters. PC 1 accounted for 44.2% of the variance, with the hydration capacity, hydration index, swelling capacity and swelling index being the main contributors. PC 2 accounted for 18.4% of the variances, with the colour parameters (L^*, a*, b*) being the major contributors. PC 3 accounted for 12.2% of the total variation, with the seed size and seed volume being the main contributors. PC 4 depended on the hydration capacity and hydration index.

Table 4.3 Summary of the factor loadings, eigenvalues and percent variations for the cooking-quality parameters assessed for 156 bambara groundnut genotypes

Parameter	PC 1	PC 2	PC 3	PC 4
Seed size	-0.373	-0.190	0.873	-0.207
Seed volume	-0.451	-0.109	0.778	-0.246
Density	0.135	-0.160	0.248	0.099
Hydration capacity	0.751	0.124	0.349	0.434
Hydration index	0.803	0.155	0.108	0.467
Swelling capacity	0.844	0.045	0.321	0.320
Swelling index	0.867	0.080	0.128	0.345
Cooking time	-0.830	0.416	0.039	0.346
EC	-0.824	0.407	0.037	0.295
Ph	0.781	-0.432	-0.055	-0.383
L*	0.478	0.784	0.042	-0.248
a*	0.236	0.721	0.087	-0.448
b*	0.438	0.816	0.044	-0.312
Texture	-0.836	0.368	0.051	0.337
Eigenvalue	6.186	2.581	1.702	1.567
Variability (%)	44.187	18.437	12.158	11.195
Cumulative %	44.187	62.624	74.782	85.978

EC: Electrical Conductivity, L*: degree of Lightness, a*: degree of green to red, b*: degree of blue to yellow

The biplot for the first two principal components is presented in Figure 4.2. The PCA biplot was constructed, with the two principal components accounting for 62.2% of the total variability. The biplot diagram highlights the strongest positive and parallel effects of texture on the cooking time, followed by the electrical conductivity (Figure 4.2). The hydration and swelling properties showed a negative effect on the cooking time (Figure 4.2).

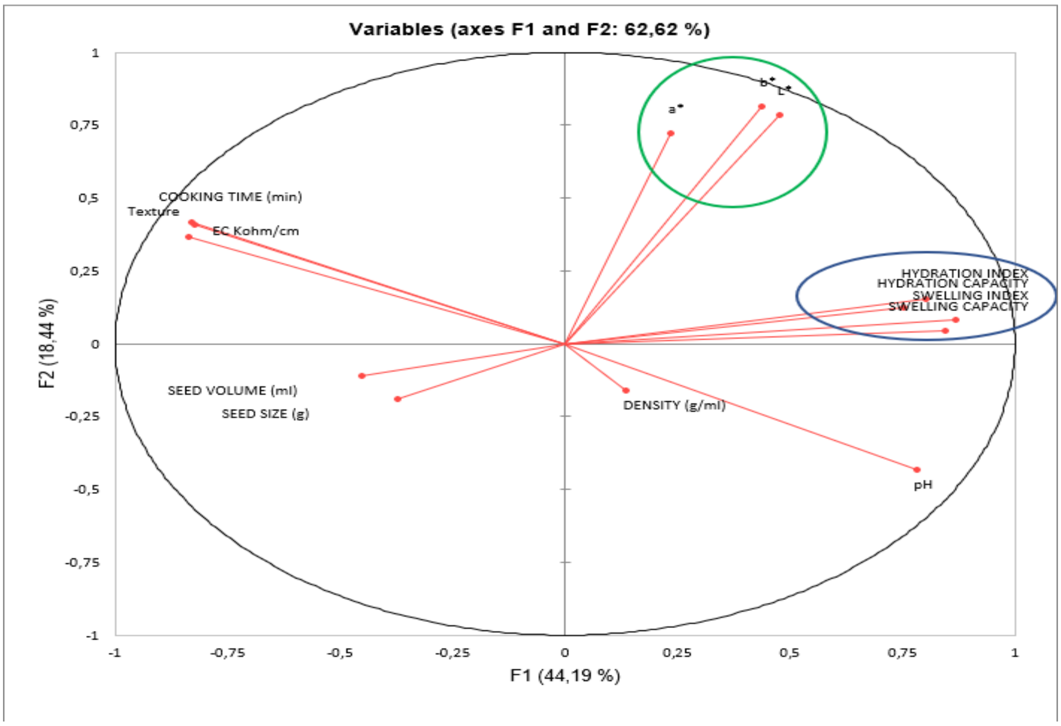

Figure 4.2 **Biplot for the first two principal components of the cooking quality parameters of the bambara groundnut genotypes**

4.5.4 Proximate composition

A paired t-test was performed to identify the statistical significance between the raw and cooked nutritional composition of BGNs. The proximate composition of the raw and cooked BGNs is presented in Tables 4.4 and 4.5. The results show that the concentrations of all analysed proximate nutrients were significantly different using a paired t-test. The protein content of raw BGN seeds ranged from 15.16 to 29.32 g/100 g. The mean protein content of cooked BGN samples was higher than that of the raw samples and was significantly different (p = 0.035) from the raw BGN samples. The genotype DodR showed the highest protein recovery after cooking (19.09-25.39 g/100 g). The fat content of raw BGN samples (3.16-5.55 g/100 g) was lower than that of cooked BGN samples (5.95-8.16 g/100 g) and was significantly different at p<0.001. The genotype Nav 4 showed the highest fat content recovery (3.16-6.77 g/100 g) after cooking. The results showed that BGN is mainly composed of Neutral Detergent Fibre (NDF), with a maximum of 46.8 and 64.09 g/100 g for the raw and cooked samples, respectively. The total mineral content (ash) of raw BGN samples was higher (4.18-5.23 g/199 g) and significantly different p<0.001 from that of the cooked samples (2.5-4.85 g/100 g). The moisture content of raw BGNs (3.65-8.45%) was significantly different p<0.001 from that of the cooked BGN samples (8.7-9.59%).

Table 4.4 Proximate composition of selected raw samples (10 fastest and 10 slowest cooking) of bambara groundnut genotypes

Genotype	Protein (g/100 g, DW)	Fat (g/100 g, DW)	NDF (g/100 g, DW)	ASH (mg/100 g, DW)	Moisture (%)
Fast cooking	**BGN lines**				
S19/Ankpa4-100-87	22.41	3.90	40.56	4.86	8.04
NAV 4	24.63	3.16	44.80	4.86	6.82
S19/Ankpa4-92-79	28.56	3.83	36.18	5.10	7.59
TIGD	28.58	3.21	38.73	4.18	6.52
IITA686/LunT-403-314	25.82	4.14	36.42	4.57	6.66
S19/Ankpa4-234-197	22.4	4.96	41.45	4.35	8.30
S19/Ankpa4-141-121	23.74	3.72	28.29	4.67	8.26
BURKINA	29.32	3,.81	31.88	5.15	6.85
ANKPA 4	25.33	3.95	34.95	5.23	7.90
DodR	21.03	5.18	34.22	4.86	7.58
	Slow cooking	**BGN lines**			
S19/Ankpa4-106-92	25.27	4.38	20.95	4.81	8.31
SONGKHLA	23.81	5.55	29.60	4.62	3.65
S19/Ankpa4-89-76	22.00	4,.36	20.07	4.98	7.26
UNISWA	20.59	5.21	23.41	4.58	7.38
S19/Ankpa4-171-143	24.95	3.95	28.13	4.90	7.73
GHC37105	22.48	4.30	28.82	4.54	7.96
DIP-C	18.82	5.52	27.68	4.25	8.45
MOQ-4	15.16	4.49	28.37	4.47	7.73
S19/Ankpa4-179-150	19.09	4.67	22.45	4.41	7.69
IITA686/LunT-334-262	24.95	4.94	16.78	4.91	7.99

Table 4.5 **Proximate composition of selected cooked samples (10 fastest and 10 slowest cooking) of bambara groundnut**

Genotype	Protein (g/100 g, DW)	Fat (g/100 g, DW)	NDF (g/100 g, DW)	ASH (mg/100 g, DW)	Moisture (%)
		Fast cooking	**BGN lines**		
S19/Ankpa4-100-87	24.95	5.95	16.78	4.85	3.65
NAV 4	25.12	6.77	18.34	3.57	3.62
S19/Ankpa4-92-79	25.95	6.42	49.25	4.33	1.82
TIGD	27.85	6.74	16.68	2.98	3.36
IITA686/LunT-403-314	25.82	6.89	56.71	3.28	3.86
S19/Ankpa4-234-197	21.94	6.89	15.47	3.52	2.73
S19/Ankpa4-141-121	24.53	6.58	58.32	3.37	2.31
BURKINA	28.94	6.64	54.75	3.33	3.43
ANKPA 4	27.24	6.18	20.05	3.46	3.47
DodR	22.54	7.48	14.23	3.46	2.62
		Slow cooking	**BGN lines**		
S19/Ankpa4-106-92	27.09	6.39	55.98	3.78	2.70
SONGKHLA	22.11	7.57	57.33	3.99	3.16
S19/Ankpa4-89-76	24.43	6.93	58.65	3.59	4.34
UNISWA	20.70	6.82	61.62	4.55	2.29
S19/Ankpa4-171-143	25.67	6.32	56.58	3.29	2.20
GHC37105	23.36	6.30	54.43	4.29	2.60
DIP-C	24.00	8.16	17.31	3.07	3.86
MOQ-4	18.23	7.30	64.09	3.40	3.15
S19/Ankpa4-179-150	25.39	7.02	59.45	2.50	2.57
IITA686/LunT-334-262	24.83	6.48	60.86	3.33	2.39
Standard deviation	**2.14**	**0.55**	**26.11**	**0.56**	**1.38**
T value	-2.28*	-19.77**	-2.17*	0.89**	14.36**

DW: Dry weight basis, **NDF**: Neutral Detergent Fibre, ** p<0.001, * <0.05

4.5.5 Mineral concentration of raw and cooked BGN samples

The mineral concentration of raw and cooked BGN samples is shown in Tables 4.6 and 4.7. All the selected nutrients that were analysed exhibited significant differences, except for phosphorous (p = 0.309) and magnesium (p = 0.22). Iron was not detected in some cooked samples. An increase in the number of nutrients after cooking was observed in zinc (2.4-3.2 mg/100 g) to (2.5-3.7 mg/100 g), sodium (0.00-0.0002 mg/100 g) to (0.002 to 0.007mg/100 g), copper (0.6-2.1 mg/100 g) to (0.9-6.3 mg/100 g) and calcium (0.0004-0.001mg/100 g) to (0.008-0.018mg/100 g). Cooking also caused a reduction in the number of nutrients, as observed in iron from (1.7-9.6 mg/100 g) to (0.00-2.9 mg/100 g), and potassium from (0.117-0.176 mg/100 g) to (0.046-0.149mg/100 g). Iron and zinc were the predominant minerals in raw BGN genotypes, ranging from 1.7-9.6 mg/100 g and 2.4-3.2 mg/100 g, respectively.

Table 4.6 Selected mineral content of the raw bambara groundnut samples of 10-fastest cooking and 10-slowest cooking genotypes (mg/100 g DW)

Genotype	Ca	Cu	Fe	K	Mg	Mn	Na	Zn	P
RAW SAMPLES				**Fast cooking BGN lines**					
S19/Ankpa4-100-87	0.005	1.200	2.600	0.159	0.015	0.900	0.001	2.400	0.044
NAV 4	0.006	1.000	2.100	0.154	0.018	0.900	0.001	2.600	0.051
S19/Ankpa4-92-79	0.009	1.500	1.700	0.160	0.019	0.600	0.002	2.800	0.059
TIGD	0.009	0.600	2.100	0.170	0.023	1.300	0.001	2.600	0.044
IITA686/LunT-403-314	0.007	0.700	2.400	0.147	0.017	0.900	0.001	2.600	0.043
S19/Ankpa4-234-197	0.006	0.800	2.400	0.151	0.018	0.900	0.001	2.400	0.044
S19/Ankpa4-141-121	0.004	0.900	2.600	0.154	0.016	0.900	0.001	2.400	0.045
BURKINA	0.001	1.100	3.000	0.146	0.022	0.900	0.001	2.600	0.059
ANKPA 4	0.005	0.900	2.800	0.176	0.015	1.100	0.001	2.600	0.051
DodR	0.004	1.000	2.800	0.174	0.018	0.900	0.001	2.600	0.037
				Slow cooking BGN lines					
S19/Ankpa4-106-92	0.006	1.200	2.800	0.159	0.019	1.100	0.001	3.000	0.057
SONGKHLA	0.005	2.100	3.000	0.158	0.016	1.100	0.001	3.200	0.045
S19/Ankpa4-89-76	0.005	0.800	4.300	0.167	0.018	0.900	0.001	2.600	0.042
UNISWA	0.005	0.700	4.600	0.150	0.017	0.900	0.001	2.600	0.038
S19/Ankpa4-171-143	0.004	0.800	2.600	0.169	0.017	1.100	0.000	2.400	0.042
GHC37105	0.005	0.800	3.000	0.152	0.017	0.900	0.001	2.400	0.044
DIP-C	0.007	0.700	2.400	0.138	0.017	0.900	0.000	2.600	0.037
MOQ-4	0.004	0.600	3.000	0.159	0.017	1.100	0.001	2.400	0.038
S19/Ankpa4-179-150	0.005	0.800	9.600	0.164	0.016	1.300	0.000	2.600	0.034
IITA686/LunT-334-262	0.006	0.800	2.600	0.173	0.018	1.100	0.002	25.000	0.048

Table 4.7 Selected mineral content of cooked bambara groundnut samples of the 10-fastest cooking and 10-slowest cooking genotypes (mg/100 g DW)

Genotype	Ca	Cu	Fe	K	Mg	Mn	Na	Zn	P
COOKED SAMPLES			**Fast**	**cooking BGN lines**					
S19/Ankpa4-100-87	0.012	1.100	2.900	0.122	0.017	1.000	0.002	2.500	0.048
NAV 4	0.013	0.900	0.000	0.115	0.017	0.600	0.002	2.900	0.049
S19/Ankpa4-92-79	0.012	2.000	0.000	0.073	0.018	0.400	0.002	2.600	0.046
TIGD	0.018	3.100	0.000	0.093	0.023	0.600	0.002	2.900	0.056
IITA686/LunT-403-314	0.017	2.100	0.000	0.046	0.015	0.600	0.002	2.500	0.047
S19/Ankpa4-234-197	0.010	1.100	0.000	0.051	0.017	0.400	0.002	2.500	0.038
S19/Ankpa4-141-121	0.009	1.500	0.000	0.095	0.015	0.400	0.002	2.500	0.044
BURKINA	0.017	1.300	0.000	0.094	0.021	0.400	0.002	2.500	0.044
ANKPA 4	0.011	1.500	0.000	0.053	0.014	0.600	0.002	2.700	0.046
Dod R	0.008	1.000	1.800	0.078	0.015	0.800	0.003	2.900	0.046
			Fast	**cooking BGN lines**					
S19/Ankpa4-106-92	0.013	1.400	2.500	0.100	0.018	1.300	0.003	3.300	0.036
SONGKHLA	0.014	3.200	2.300	0.060	0.018	1.000	0.003	3.500	0.049
S19/Ankpa4-89-76	0.015	2.400	1.600	0.114	0.017	1.000	0.007	3.100	0.042
UNISWA	0.013	6.300	1.400	0.075	0.018	1.000	0.004	3.700	0.044
S19/Ankpa4-171-143	0.015	1.600	1.900	0.149	0.016	1.200	0.006	2.900	0.037
GHC37105	0.012	2.800	1.900	0.076	0.018	0.800	0.004	2.900	0.040
DIP-C	0.011	1.700	1.400	0.126	0.016	0.800	0.005	3.100	0.042
MOQ-4	0.011	1.700	1.400	0.075	0.014	0.800	0.002	2.700	0.039
S19/Ankpa4-179-150	0.014	1.300	2.300	0.082	0.015	1.200	0.003	2.900	0.035
IITA686/LunT-334-262	0.010	1.300	1.700	0.050	0.015	1.000	0.002	2.900	0.034
Standard deviation	**0.002**	**1.280**	**1.590**	**0.030**	**0.001**	**0.250**	**0.002**	**0.280**	**0.009**
t value	-13.200**	-3.540*	5.520**	9.530**	2.490$^{n.s}$	3.360*	-5.120**	-4.410**	1.040$^{n.}$

DW: Dry Weight basis, Ca: calcium, Cu: copper, Fe: Iron, K: potassium, Mg: magnesium, Mn: manganese, Na: sodium, Zn: zinc, P: phosphorous, ** p<0.001, * p<0.005, n.s p>0.05

4.6 Discussion

The grain quality is a complex quantitative trait that is determined by its physical appearance, milling properties, nutritional composition and cooking quality (Hori, 2022). Grain quality is the most important attribute of legumes, after yield and drought tolerance. Assessing the grain quality is an important pre-breeding task for identifying lines with a better grain quality. A knowledge of the traits correlated to grain quality will enable the precise and effective selection of parental material to develop consumer-preferred varieties. The seed size showed a highly significant and positive correlation with the seed volume. Similar results were reported for *Cicer arietinum* (Geethanjali et al., 2018) and for *Phaseolus nungo* L.(Singh et al., 2004). The swelling capacity and the swelling index were significantly positively correlated to the degree of lightness (L), r = 0.367 and r = 0.389, respectively. These results showed that the light-coloured genotypes swelled more than the dark-coloured genotypes.

The hydration capacity was positively correlated to the hydration index (r = 0.94), the swelling capacity (r = 0.83) and the swelling index (r = 0.76). The present results agree with those reported by Singh et al., (2004), who reported a positive correlation of the hydration capacity with the hydration index (r = 0.64), swelling capacity (r = 0.66) and swelling index (r = 0.612). Both the hydration and swelling properties were significantly correlated to the cooking time. These characteristics could be used indirectly for selecting genotypes based on the cooking time. This method is easy, rapid, and cheap and can be adopted to phenotype-allied legume species with similar challenges.

The seed size was also significantly correlated with cooking, although the association was weak. A similar positive correlation between the cooking time and seed size were reported by Singh et al. (2010). This could be due to an increase in the distance of water penetration to the innermost parts of the seeds (Kaur et al., 2004). The cooking time was significantly and positively correlated with texture (r = 0.96), and a similar observation was reported by Bassett et al. (2021). These results indicate that BGN genotypes that took longer to cook had a firmer texture, which implies that fast-cooking genotypes also give a better texture.

The differences in the nutritional composition of legumes are attributed to the genotype, the growing conditions, as well as the techniques used for the nutrition analysis (Boye et al., 2010). The protein concentration of all BGN genotypes increased after soaking and cooking, and these findings were in line with those of Wang et al., (2010) for common beans and chickpeas. This increase is attributed to the denaturing of some anti-nutritional factors, which results in the release of nutrients (Abdulsalami & Sheriff, 2010). This trend could also be due to the loss of soluble solids, which increases the protein concentration (Wang et al., 2010). Protein-energy malnutrition is one of the major health problems in developing countries (Müller & Krawinkel, 2005). Many individuals from economically disadvantaged households within developing countries cannot afford nutritious foods that form part of a diversified diet. Their diets lack animal protein, which is expensive, and these households rely heavily on starch-based foods (Altman et al., 2010, Statistics SA.,2018., Govender et al.,2019). When starch-based foods such as maize are predominately consumed, it results in poor quality proteins from the diet. As mentioned earlier, BGN is a good source of protein and thus will be a good complementary protein when consumed with starch-based foods such as maize. Furthermore, it contains the essential amino acid methionine, emphasising that this crop is nutritionally complete (Linnemann & Azam-Ali 1993, Mwale & Massawe, 2007; Stone et al, 2011; Litchtouse, 2016; Govender et al, 2019). Therefore, the results of this study indicate that BGN seeds could be used as an alternative cheap good quality protein source, due to their considerably high protein content, and could help reduce protein deficiency in developing countries.

Fats are important for good health as they provide energy and assist in the absorption of vitamins, as well as in hormone function (Bennasir et al., 2010). However, due to misunderstandings about their role in the human body, less attention has been given to them in the food systems (Bajželj et al., 2021). Soaking and cooking BGN seeds significantly increased the fat content ($p<0.001$), which agrees with the findings of Mazahib et al. (2013). Although the fat content increased significantly after cooking, it remains within the range of the Recommended Dietary Allowance of 20-35 g/100 g for adults (FAO-WHO,2010).

Ash is the measurement of the total mineral content and inorganic matter biomass (Bilge et al., 2016). The determination of ash is an important step in a mineral composition analysis. The results of this study indicated that the total mineral content (ash) decreased with cooking, which is in agreement with those of Shanono & Muhammad (2015); however, they contrasted with the observations of Mazahib et al. (2013). Thermal treatments have been reported to enhance and decrease the bio-accessibility of different minerals (Platel & Srinivasan, 2016). The cooking process does not destroy mineral elements due to their heat stability; rather, they are leached into the cooking water (Wang et al., 2010). Soaking and cooking resulted in significant differences in the availability of selected mineral elements, except for magnesium and phosphorous. While there was a significant increase in calcium, copper, zinc and sodium, there was a significant decrease in iron. The effects of cooking on phosphorous agreed with the results of Ndidi et al. (2014), who found an insignificant difference in the phosphorous content after boiling BGNs. These results indicated that soaking and cooking increased the calcium content of the BGN samples; conversely, those of Mazahib et al. (2013) found a decrease in the calcium content after cooking. Calcium plays an important role in the formation of bone and teeth, nerve impulses and muscle contradictions (Pravina et al., 20 13). After cooking, S19/Ankpa 4-171-143 showed the highest calcium recovery (0.004-0.015 mg/100 g). The iron content significantly decreased after cooking, which could be due to leaching. The current results agree with those of Mazahib et al. (2013), whereas Omoikhoje (2008) found an increase in the iron content with cooking. Iron biofortification can be done to improve the iron content of BGNs. As alluded to, iron deficiency is a common micronutrient deficiency. The iron content in BGN could be enhanced by consuming BGN with iron-rich foods and breeding crops that contain more iron.

The moisture content of dried legumes is important for determining their shelf-life, product quality and processing techniques (McCurdy et al., 1980). The moisture content of BGN genotypes was found to decrease after cooking. These findings are contrary to the results of (Shanono & Muhammad, 2015), who observed a significant increase in the moisture content after cooking. This could be due to use of different genotypes in these studies. The moisture content of BGN samples after cooking was within the range of recommended values (3-8%) for the long storage of legumes.

Micronutrient malnutrition is a serious problem worldwide, affecting approximately two billion people (WHO, 2019). It is mainly caused by the unavailability and inaccessibility of food, as well as the peoples' food choices. Three main strategies are used to address this problem, namely, food fortification, supplementation, and dietary diversity (Oelofse, 2001).

4.7 Conclusion

This study provided information on the cooking quality traits, proximate composition, and mineral element content of BGN genotypes. The proximate and mineral composition analyses indicated a significant difference in the nutritional value of raw and cooked BGN samples. The genotype S19/Ankpa 4-100 had the shortest cooking time and a relatively high iron, potassium, manganese, and magnesium content. The Burkina genotype had the highest protein content after cooking, while Dip-C had the highest fat content. The highest amount of NDF was observed in Moq-4, and Uniswa had the highest iron content. These genotypes could be utilised in breeding for their short cooking times and a high protein content. The seed size was correlated to the cooking time and could be used as a quick indicator of the cooking time. However, selecting a BGN for short cooking, based on seed size, could compromise the yield. Selection indices can be used to obtain a more significant genetic gain when selecting negatively-correlated traits.

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CHAPTER 5
SUMMARY, IMPLICATIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter presents an overview of the research study by highlighting its objectives, the main findings and the recommendations. BGN is a crop that is widely grown worldwide, and it is used for food, animal fodder and medicinal purposes. It has the potential to be used as a reliable supplementary food due to its high nutritional composition. However, breeding for improved BGN genotypes is limited due to its narrow genetic base. Morphological characterisation is important and it is a prerequisite for plant breeding programmes, in order to develop superior varieties with desirable characteristics. Although agro-morphological characterisation is critical in plant breeding, there is a need to explore the cooking quality and nutritional composition of BGN genotypes. Therefore, the aims and objectives of this study were as follows:

Aims and study objectives

This study aimed to identify BGN genotypes with desirable agro-morphological traits and cooking-quality properties, in order to recommend lines that can be used for future plant breeding programmes.

Specific objectives

The specific objectives of the study were:

i) to evaluate the genetic diversity among BGN Recombinant Inbred Lines (RILs), using agronomic and morphological traits for future plant breeding programmes; and

ii) to assess the cooking quality properties and nutritional composition of selected BGN RILs.

5.2 The Findings from the Literature

In the literature, the HTC trait is the major factor that limits the utilisation of BGNs. The storage conditions, the seed age and the genetic make-up are the main reasons for the development of this trait. The use of a salt solution is the most common method used to reduce the cooking time of legumes. However, there is limited information regarding the impact of the different strategies used to manage the nutritional composition and cooking quality of this trait. There is also a paucity of information on the sustainable management of HTC traits. Breeding for a short cooking time in BGN has not been given much attention.

5.3 Summary of Research Findings

The first part of the study identified the agronomic and phenotypic differences among BGN genotypes. Significant differences ($p<0.050$) were exhibited in the agro-morphological traits of BGN, except for the canopy width, which indicated that the genotypes have important variations for selecting parental material in future plant breeding programmes. A correlation analysis indicated that the days to emergence, the days to 50% flowering, the plant height and petiole length were significantly and positively correlated to the grain yield per plant. These associations show the potential of selecting those traits, in order to improve the grain yield.

The second part of the study evaluated the physical, cooking and nutritional properties of BGN. The cooking time ranged from 40 to 147 minutes, with S19/Ankpa 4-100-87 having the shortest cooking time. There was a significant difference in the proximate and mineral content composition between the raw and cooked BGN samples. In general, cooking improved the nutritional composition of the selected BGNs. The hydration properties, swelling properties, degree of lightness and pH were significantly negatively correlated to the cooking time, while the electrical conductivity, seed size and texture showed a significantly positive correlation to the cooking time.

5.4 Recommendations

5.4.1 Study limitations

- Due to the financial constraints, a nutritional analysis was only performed on selected BGNs, and an Amino Acid (AA) analysis could not be carried out.
- This study only focused on the agro-morphological traits affected by the genotype × environment interactions, which limited the genetic differences at a molecular level.

5.4.2 Implications for future research

- A nutritional analysis should be done on all BGN genotypes, in order to increase the sampling population.
- Consumer acceptability studies should be conducted.

5.4.3 Recommendations for the improvement of the study

A further analysis of the genetic diversity among BGN genotypes, using molecular markers, will provide detailed information on the genetic control of traits. Multiple location experiments should be conducted to determine the effects of the genotype × environment interactions. A nutritional analysis of all the genotypes, as well as further studies on the nutrient quality, accessibility and digestibility, should be undertaken. BGN seeds could also be used as supplementary feed in feeding schemes because of their high nutritional composition.

APPENDIX A: TREATMENT MEANS

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
100SB16ANAM-C	41.80	13.40	55.80	65.2	18.18	2.54	22.6	11.8
20ACC118CIVB	39.20	20.60	72.60	17.2	6.2	2.32	26.4	6.4
ANKPA 4	43.20	21.80	77.20	7.60	3.06	2.78	23.8	18.6
BURKINA	41.60	15.80	58.60	7.60	4.4	4.1	22.8	9.4
DIP-C	49.40	21.40	78.40	34.80	11.6	1.58	28.2	19.2
DOD R	42.40	19.60	71.20	27.2	8.66	2.3	25.2	14.6
EXSOCOTO	42.80	18.20	66.60	36.8	10.34	2.36	23.8	14.4
GHC37105	40.20	22.60	80.60	54.4	14.54	0.86	29.8	22.8
IITA686	37.20	22.20	79.00	52.8	17.5	3.24	29.8	19.8
11TA686/LUNT-257-216	26.48	22.76	79.86	63.44	16.87	0.74	28.9	21.09
IITA686/LUNT-258-217	46.48	14.76	55.86	0.00	1.37	2.64	21.9	8.09
IITA686-258-217	35.48	14.76	56.86	1.44	7.37	2.64	21.9	10.09
IITA686/LUNT-260-219	49.79	19.53	71.29	30.6	7.82	0.88	25.25	12.39
IITA686/LUNT-261-220	52.79	19.53	71.29	42.6	10.82	3.48	25.25	13.39
IITA686/LUNT-262-221	49.79	18.53	68.29	28.6	10.02	2.58	24.25	11.39
IITA686/LUNT-264-222	30.79	16.53	79.29	10.6	13.32	1.08	22.25	7.39
IITA687/LUNT-265-223	48.48	19.76	69.86	35.44	13.37	2.54	24.9	14.09
IITA686/LUNT-266-224	57.48	19.76	69.86	3.44	2.67	1.94	24.9	15.09
IITA686/LUNT-269-225	35.74	17.49	64.47	35.51	9.23	3.53	24.56	10.16
IITA686/LUNT-271-226	27.48	16.72	56.86	7.44	4.07	3.74	23.9	13.09
IITA686/LUNT-274-227	38.74	12.49	50.47	27.51	6.53	5.03	18.56	6.18
IITA686/LUNT-280-228	43.74	17.49	48.47	27.51	6.53	3.33	16.56	6.16
IITA686/LUNT-283-229	63.74	12.49	50.47	27.51	6.53	5.03	16.56	5.16

TREATMENT	**CW**	**DE**	**DF**	**GY**	**GYP**	**IL**	**PH**	**PL**
IITA686/LUNT-284-230	39.25	14.17	53.09	13.53	5.54	4.56	23.86	6.59
IITA686/LUNT-289-231	55.25	14.17	54.09	0.00	0.00	37.06	21.86	9.59
IITA686/LUNT-291-232	27.74	20.06	71.29	6.91	1.94	1.18	26.43	15.77
IITA686/LUNT-292-233	40.74	22.06	78.29	44.91	11.44	2.48	28.43	19.59
IITA686/LUNT-293-234	56.25	22.17	78.09	0.00	0.84	3.96	28.86	19.59
IITA686/LUNT-295-235	35.25	14.17	52.09	25.53	12.84	4.86	29.86	6.59
IITA686/LUNT-296-236	53.48	21.76	78.86	29.44	6.57	1.24	27.9	19.09
IITA686/LUNT-297-237	28.74	21.06	77.29	10.91	5.94	2.78	26.43	19.77
IITA686/LunT-298-238	37.74	20.06	71.29	10.91	3.94	3.48	26.43	15.77
IITA686/LunT-300-239	48.79	16.53	77.29	62.60	12.52	1.78	27.25	18.39
IITA686/LunT-301-240	62.25	21.17	75.09	59.53	13.84	2.86	26.86	9.59
IITA686/LunT-302-241	49.74	14.49	55.47	27.51	6.53	4.43	20.56	6.16
IITA686/LunT-303-242	48.74	13.49	53.47	29.51	8.53	4.63	21.56	8.16
IITA686/LunT-305-243	30.79	21.53	78.29	58.60	20.02	1.98	21.25	21.39
IITA686/LunT-306-244	37.79	12.53	50.29	8.60	3.32	4.88	19.25	6.39
IITA686/LunT-309-245	57.74	14.06	55.29	22.91	7.24	4.88	21.43	8.77
IITA686/LunT-310-246	24.24	16.17	55.09	0.00	14.84	2.96	20.86	7.59
IITA686/LunT-312-247	34.74	15.49	57.47	49.51	17.53	4.43	22.56	10.16
IITA686/LunT-313-248	64.48	18.76	68.86	9.44	7.37	2.94	24.90	14.09
IITA686/LunT-314-249	45.25	19.17	68.09	9.53	1.34	3.06	23.86	12.59
IITA686/LunT-316-250	47.74	19.06	68.29	6.91	1.94	1.18	24.43	18.77
IITA686/LunT-318-251	53.25	15.17	56.09	1.53	5.84	1.36	22.86	11.59
IITA686/LunT-319-252	55.25	20.17	69.09	1.53	1.54	2.16	21.86	13.59
IITA686/LunT-320-253	36.74	23.06	81.29	6.91	1.94	1.68	30.43	22.77
IITA686/LunT-321-254	61.74	17.49	63.47	53.51	19.53	6.63	24.54	13.60

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
IITA686/LunT-322-255	39.74	22.06	79.29	18.91	5.94	2.08	23.43	21.77
IITA686/LunT-324-256	49.79	21.53	77.27	24.60	6.32	2.78	28.25	18.39
IITA686/LunT-326-257	44.74	16.49	60.47	39.51	10.53	3.83	23.76	12.16
IITA686/LunT-327-258	24.74	18.06	65.29	30.91	9.94	1.58	24.43	12.77
IITA686/LunT-330-259	44.48	17.76	77.86	45.44	7.77	2.04	28.90	21.09
IITA686/LunT-332-260	40.25	22.17	77.09	0.00	4.84	2.16	27.86	18.59
IITA686/LunT-333-261	41.74	13.49	54.47	27.51	6.53	4.53	20.56	12.16
IITA686/LunT-334-262	43.25	15.17	55.09	19.53	14.84	2.06	19.86	9.59
IITA686/LunT-335-263	38.74	14.49	54.47	31.51	19.53	1.53	22.56	7.16
IITA686/LunT-337-264	39.79	15.53	58.29	32.6	8.32	4.08	22.25	10.39
IITA686/LunT-338-265	39.48	19.76	19.86	0.00	1.37	3.34	24.9	13.09
IITA686/LunT-340-267	33.74	13.29	51.47	27.51	6.53	4.83	18.56	6.16
IITA686/LunT-341-268	31.48	22.76	79.86	59.44	12.57	1.44	30.90	22.09
IITA686/LunT-344-269	36.74	23.06	82.29	26.91	8.64	0.98	33.43	24.77
IITA686/LunT-345-270	53.48	16.76	59.86	0.00	1.37	3.24	22.90	11.09
IITA686/LunT-348-271	42.79	11.53	78.29	76.60	19.32	3.78	29.75	21.39
IITA686/LunT-350-272	47.74	13.49	53.47	27.51	6.53	4.63	25.56	6.16
IITA686/LunT-351-273	61.76	19.49	69.47	31.51	8.53	3.03	25.56	16.16
IITA686/LunT-352-274	50.74	18.06	64.29	8.91	3.94	4.28	23.43	12.77
IITA686/LunT-353-275	36.74	20.49	27.47	29.53	8.53	2.83	25.56	10.16
IITA686/LunT-354-276	38.79	22.53	79.29	8.60	5.32	2.88	29.25	21.39
IITA686/LunT-356-277	45.79	22.53	81.29	26.60	9.32	3.48	32.25	22.39
IITA686/LunT-357-278	31.74	19.06	69.29	36.91	11.94	3.38	28.43	14.77
IITA686/LunT-359-279	47.74	22.06	77.29	12.91	3.94	4.18	27.43	17.77
IITA686/LunT-360-280	41.25	17.17	68.09	50.53	7.84	3.76	24.86	13.59

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
IITA686/LunT-362-281	47.25	17.17	61.09	29.53	6.34	3.96	22.86	10.59
IITA686/LunT-363-282	54.74	20.49	72.49	27.51	6.53	2.73	25.56	14.16
IITA686/LunT-364-283	42.74	12.06	70.29	16.21	6.94	2.48	21.43	14.77
IITA686/LunT-365-284	25.25	22.17	78.09	7.53	8.84	2.16	28.86	20.59
IITA686/LunT-366-285	47.25	15.17	78.09	7.53	0.84	2.36	28.86	20.59
IITA686/LunT-367-286	28.74	18.49	81.47	69.51	48.53	0.93	29.54	20.16
IITA686/LunT-369-287	40.74	20.06	72.29	16.91	4.64	2.98	26.43	15.77
IITA686/LunT-370-288	29.79	21.53	76.29	54.60	13.86	3.38	26.25	11.39
IITA686/LunT-370-289	62.25	20.17	69.09	0.00	2.84	3.16	24.86	15.59
IITA686/LunT-373-290	28.25	12.17	76.09	0.00	1.84	2.96	19.86	7.59
IITA686/LunT-374-291	40.74	20.06	71.29	24.91	6.44	5.28	26.43	15.77
IITA686/LunT-375-292	50.74	16.49	58.47	33.51	12.43	4.13	22.56	11.16
IITA686/LunT-377-293	49.74	14.49	59.47	29.51	8.53	41.83	23.56	11.16
IITA686/LunT-378-294	44.79	20.53	74.29	12.60	7.32	2.38	26.25	14.39
IITA686/LunT-379-295	62.74	22.06	80.29	56.91	11.93	3.38	21.43	20.77
IITA686/LunT-381-296	63.74	19.49	69.47	29.51	8.53	3.03	21.54	13.16
IITA686/LunT-384-297	42.48	16.76	60.86	0.00	0.00	3.74	23.90	12.09
IITA686/LunT-385-298	37.74	15.59	58.47	29.51	5.53	4.13	22.56	8.16
IITA686/LunT-386-299	31.79	21.53	78.29	58.60	20.02	2.08	27.25	18.39
IITA686/LunT-386-300	48.48	16.67	62.86	43.44	16.07	3.54	22.90	12.09
IITA686/LunT-387-301	64.48	16.76	58.86	25.44	7.37	0.74	22.90	12.09
IITA686/LunT-388-302	37.25	15.17	56.09	13.53	0.44	4.56	21.86	9.59
IITA686/LunT-389-303	34.74	23.49	82.47	41.51	11.23	3.63	31.56	24.16
IITA686/LunT-390-304	32.25	14.17	52.09	55.53	8.84	4.06	19.86	6.59
IITA686/LunT-392-305	41.79	22.53	80.29	12.60	7.32	1.58	29.25	20.39

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
IITA686/LunT-394-306	52.48	19.76	70.86	1.44	2.07	1.14	25.90	12.09
IITA686/LunT-395-307	42.25	20.17	71.09	0.00	4.84	5.06	25.86	14.59
IITA686/LunT-396-308	60.74	20.49	71.47	31.51	8.53	1.13	25.56	14.16
IITA686/LunT-397-309	26.74	17.49	63.47	35.51	10.53	1.23	24.56	10.16
IITA686/LunT-398-310	44.48	22.76	79.86	45.44	25.37	0.74	29.90	22.09
IITA686/LunT-399-311	61.79	14.53	80.29	16.06	6.02	2.88	30.25	21.39
IITA686/LunT-400-312	27.74	18.06	67.29	26.91	11.94	3.58	23.43	11.77
IITA686/LunT-402-313	35.79	16.53	61.29	22.60	8.02	4.78	23.23	11.39
IITA686/LunT-403-314	31.25	20.17	72.09	5.53	0.34	0.86	25.86	11.59
IITA686/LunT-403-315	33.48	16.76	59.86	64.44	17.37	2.34	22.90	20.09
IITA686/LunT-404-316	43.79	22.53	79.29	2.06	2.32	4.38	29.25	18.39
IITA686/LunT-407-317	41.79	22.53	82.29	8.86	5.32	2.58	30.25	21.39
IITA686/LunT-408-318	48.48	16.76	60.86	37.44	8.17	2.74	22.90	11.09
IITA686/LunT-411-319	46.25	12.17	73.09	0.00	0.00	2.66	26.86	16.59
IITA686/LunT-412-320	37.74	18.49	65.47	27.51	6.53	3.43	20.56	12.16
IITA686/LunT-415-321	47.25	15.17	55.09	73.53	25.54	1.66	20.86	7.59
IITA686/LunT-416-322	26.79	12.53	68.29	4.60	3.32	3.08	24.25	11.39
IITA686/LunT-417-323	42.79	16.53	78.29	24.60	4.92	2.18	27.25	19.39
IITA686/LunT-419-324	38.25	19.17	67.09	73.53	17.34	3.26	22.86	11.59
IITA686/LunT-420-325	44.25	17.17	60.09	17.53	3.34	2.68	31.25	18.39
IITA686/LunT-421-326	54.79	22.53	82.29	0.00	1.32	2.68	31.25	18.39
IITA686/LunT-422-327	49.74	23.06	80.29	12.91	7.94	0.98	30.43	22.77
IITA686/LunT-423-328	55.25	15.17	54.09	15.53	12.84	4.38	26.43	15.77
IITA686/LunT-426-329	35.74	20.06	71.29	44.91	11.44	4.38	26.43	15.77
IITA686/LunT-427-330	47.79	15.53	74.29	40.06	14.02	3.88	26.25	15.39

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
IITA686/LunT-428-331	56.74	19.49	82.47	27.51	6.53	0.73	30.56	22.16
IITA686/LunT-429-332	54.79	21.53	78.29	10.06	4.02	1.98	28.25	16.39
IITA686/LunT-430-333	42.74	20.06	70.29	6.91	1.94	2.58	25.43	15.77
IITA686/LunT-431-334	39.74	20.49	73.47	31.51	10.53	2.63	26.56	15.16
IITA686/LunT-432-335	32.79	21.53	76.29	10.60	6.32	2.28	27.25	16.39
IITA686/LunT-433-336	27.74	17.06	61.29	48.91	22.94	3.08	23.43	11.77
IITA686/LunT-434-337	50.74	23.06	83.29	30.91	7.94	3.38	32.43	24.77
IITA686/LunT-436-338	50.74	15.06	56.29	18.91	7.94	4.68	22.43	10.77
IITA686/LunT-437-339	34.48	22.76	79.86	0.00	1.37	6.64	30.9	12.09
IITA686/LunT-438-340	38.48	19.76	70.86	115.44	23.77	2.64	25.9	15.09
IITA686/LunT-439-341	47.79	20.53	75.29	0.60	3.32	3.18	26.25	17.39
IITA686/LunT-440-342	48.74	14.06	53.29	6.91	1.94	4.98	24.43	9.77
IITA686/LunT-441-343	34.48	20.76	76.86	19.44	8.07	2.14	27.90	19.09
IITA686/LunT-442-344	26.74	26.03	71.29	16.91	6.94	3.18	26.43	15.77
IITA686/LunT-443-345	24.79	12.53	51.29	10.60	4.02	4.78	20.25	7.39
IITA686/LunT-444-346	63.78	19.49	69.47	71.51	15.33	2.93	25.56	15.16
KANO2	49.80	17.40	62.40	16.00	5.46	3.42	24.00	12.80
KENYA CAPSTONE	38.80	22.20	80.60	47.20	12.10	1.84	31.00	21.40
LUN T	37.60	20.60	73.40	23.20	7.76	3.06	25.60	15.20
MOQ-4	47.80	20.60	74.80	96.20	4.68	4.30	26.00	17.60
NAV 4	39.40	19.80	71.40	16.80	6.66	3.20	25.60	14.20
PONG-BR	39.00	21.00	73.80	61.20	19.76	4.40	27.20	21.40
PONG-CR	37.60	14.80	63.20	61.60	15.02	4.06	26.00	17.80
S19	52.20	19.60	71.00	45.20	17.52	5.16	22.20	13.80
S19/Ankpa4-1-1	47.25	22.17	79.07	0.00	0.84	5.16	22.20	13.80

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-10-9	28.25	13.17	51.09	0.00	0.00	4.06	20.86	8.59
S19/Ankpa4-100-87	33.79	14.53	55.29	26.60	6.82	3.78	21.25	8.39
S19/Ankpa4-101-88	42.79	12.53	51.29	4.60	7.32	4.28	19.25	6.39
S19/Ankpa4-102-89	46.25	13.17	52.09	0.00	0.00	2.76	19.86	7.59
S19/Ankpa4-103-90	44.74	17.06	60.29	26.91	8.64	1.88	24.43	14.77
S19/Ankpa4-104-91	54.79	14.53	79.29	24.60	6.32	3.48	28.25	21.38
S19/Ankpa4-106-92	33.79	16.63	61.29	38.60	7.72	4.98	22.25	10.39
S19/Ankpa4-108-93	30.48	14.76	76.86	83.44	29.37	3.44	27.90	19.09
S19/Ankpa4-11-10	38.74	17.06	60.29	18.91	13.94	5.08	23.43	12.77
S19/Ankpa4-110-94	45.79	19.53	70.29	0.00	1.32	2.78	25.25	16.39
S19/Ankpa4-111-95	24.79	16.53	60.29	68.60	5.82	1.68	22.25	9.39
S19/Ankpa4-112-96	41.79	16.53	61.29	56.60	14.32	2.38	23.25	9.39
S19/Ankpa4-113-97	45.74	14.06	55.29	14.91	5.94	2.58	25.43	11.77
S19/Ankpa4-114-98	41.79	13.53	62.29	0.00	1.32	3.58	23.25	11.39
S19/Ankpa4-115-99	36.25	20.17	69.09	7.53	8.84	8.26	24.86	15.59
S19/Ankpa4-116-100	30.48	16.76	59.86	0.00	2.37	4.64	22.90	7.09
S19/Ankpa4-117-101	41.79	20.53	75.29	8.60	5.32	3.48	26.25	15.39
S19/Ankpa4-12-11	31.74	22.06	79.29	66.91	21.94	2.08	29.43	21.77
S19/Ankpa4-120-103	54.74	20.49	71.47	31.51	10.53	2.93	25.56	10.16
S19/Ankpa4-122-104	54.48	20.76	76.86	43.44	16.07	3.34	27.90	20.09
S19/Ankpa4-123-105	53.79	16.53	62.29	14.60	5.32	2.88	23.25	9.39
S19/Ankpa4-124-106	49.48	19.76	69.86	47.44	10.17	1.94	24.90	14.09
S19/Ankpa4-125-107	37.48	21.76	76.86	25.44	7.37	2.14	27.90	19.09
S19/Ankpa4-126-108	37.74	17.06	63.29	6.91	6.94	4.08	24.43	11.77
S19/Ankpa4-127-109	51.48	20.76	55.86	13.44	6.07	3.24	23.09	11.09

TREATMENT	**CW**	**DE**	**DF**	**GY**	**GYP**	**IL**	**PH**	**PL**
S19/Ankpa4-128-110	24.79	13.53	53.29	22.60	12.32	3.08	21.25	10.39
S19/Ankpa4-129-111	34.74	13.49	53.47	27.51	6.53	4.53	20.56	8.16
S19/Ankpa4-13-12	24.25	21.17	72.09	63.53	10.44	3.66	26.86	17.59
S19/Ankpa4-130-112	26.25	23.17	79.09	59.53	13.84	1.06	27.86	22.59
S19/Ankpa4-131-113	60.74	15.06	50.29	28.91	1.94	5.48	19.43	6.77
S19/Ankpa4-133-114	23.79	21.53	77.29	26.60	6.82	2.38	28.25	19.39
S19/Ankpa4-134-115	50.48	19.76	69.86	7.44	4.07	3.24	24.90	14.09
S19/Ankpa4-136-116	50.25	17.70	59.09	27.53	10.14	56.16	22.86	11.59
S19/Ankpa4-137-117	50.48	16.76	59.86	29.44	6.57	3.64	22.90	12.09
S19/Ankpa4-138-118	52.74	17.49	61.47	31.51	8.53	3.73	23.56	11.16
S19/Ankpa4-139-119	54.79	18.53	67.29	48.60	16.62	1.18	24.25	13.39
S19/Ankpa4-14-13	44.74	16.49	60.47	41.51	11.23	1.23	23.56	11.16
S19/Ankpa4-140-120	62.74	18.06	65.29	6.91	1.94	0.98	24.43	13.77
S19/Ankpa4-141-121	41.79	21.53	78.29	94.60	32.02	3.28	27.25	19.39
S19/Ankpa4-142-122	56.74	14.06	57.29	16.91	6.94	4.58	22.43	10.77
S19/Ankpa4-143-123	34.79	22.53	79.29	40.60	3.12	1.58	28.24	19.39
S19/Ankpa4-145-124	30.74	17.06	61.29	12.91	4.94	4.08	23.43	11.77
S19/Ankpa4-146-125	52.79	19.53	69.29	2.60	2.32	2.98	24.25	11.39
S19/Ankpa4-147-126	26.74	14.49	57.47	35.51	9.23	1.23	22.56	10.16
S19/Ankpa4-148-127	46.76	19.53	69.29	70.60	17.82	2.88	24.25	13.39
S19/Ankpa4-15-14	54.74	13.06	59.29	24.91	10.94	1.08	19.43	6.77
S19/Ankpa4-150-128	58.48	16.76	73.86	13.44	4.37	1.44	26.90	12.09
S19/Ankpa4-151-129	31.74	13.06	50.29	30.91	9.94	5.38	19.43	6.77
S19/Ankpa4-152-130	56.74	20.49	72.47	33.51	12.53	2.83	26.56	12.16
S19/Ankpa4-154-131	35.48	14.76	55.86	0.00	1.37	3.74	21.90	9.09

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-156-132	37.74	16.49	59.47	29.51	8.53	5.03	23.56	10.16
S19/Ankpa4-157-133	47.74	16.06	59.29	28.91	9.24	4.28	22.43	13.77
S19/Ankpa4-158-134	43.74	16.06	59.29	48.91	10.34	4.58	24.43	12.77
S19/Ankpa4-159-135	55.74	13.06	52.29	18.91	7.94	5.28	20.43	8.77
S19/Ankpa4-16-15	36.79	19.53	71.29	10.60	4.02	1.58	25.25	14.39
S19/Ankpa4-161-136	56.74	20.06	71.29	42.91	10.94	2.98	26.43	15.77
S19/Ankpa4-162-137	27.25	14.17	52.09	0.00	0.00	0.96	18.86	6.59
S19/Ankpa4-163-138	44.48	19.76	70.86	0.00	3.37	2.74	25.90	15.09
S19/Ankpa4-164-139	4.74	15.06	50.29	6.91	1.94	5.18	19.43	6.77
S19/Ankpa4-165-140	35.79	16.53	59.29	2.60	2.32	1.18	22.25	11.39
S19/Ankpa4-167-141	34.74	17.49	61.47	33.51	9.53	2.13	23.56	9.16
S19/Ankpa4-17-16	57.48	19.76	70.84	27.44	10.67	0.94	25.90	15.09
S19/Ankpa4-170-142	43.74	17.06	61.29	26.91	8.64	2.88	23.43	12.77
S19/Ankpa4-171-143	46.74	22.06	80.29	62.91	7.94	1.18	29.43	20.77
S19/Ankpa4-172-144	52.74	20.06	73.29	30.91	9.94	4.88	26.43	17.77
S19/Ankpa4-173-145	32.79	13.53	53.29	0.00	1.32	3.58	21.25	7.39
S19/Ankpa4-175-146	27.25	17.70	60.09	1.53	0.00	3.96	22.86	10.59
S19/Ankpa4-176-147	42.25	16.17	58.09	15.53	6.14	3.76	21.86	10.59
S19/Ankpa4-177-148	37.74	12.49	51.47	33.51	12.53	3.13	26.56	6.16
S19/Ankpa4-178-149	34.79	16.53	61.29	0.00	0.00	3.48	23.25	11.39
S19/Ankpa4-179-150	39.48	16.76	60.86	43.44	24.37	3.74	22.90	12.09
S19/Ankpa4-18-17	39.25	23.17	39.09	73.53	17.34	0.76	30.86	22.59
S19/Ankpa4-180-151	40.48	19.76	71.86	7.44	6.37	4.84	24.90	14.09
S19/Ankpa4-181-152	27.25	19.17	68.09	0.00	0.00	4.26	23.86	11.59
S19/Ankpa4-182-153	37.79	19.53	72.29	82.60	20.82	2.68	25.25	13.39

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-183-154	56.74	16.06	58.29	18.91	7.94	4.38	23.43	12.77
S19/Ankpa4-184-155	37.25	21.17	76.09	0.00	0.00	3.96	27.86	17.59
S19/Ankpa4-185-156	33.48	15.76	58.86	1.44	2.07	3.14	23.90	12.09
S19/Ankpa4-186-157	33.48	16.76	59.86	19.44	4.57	3.84	22.90	14.09
S19/Ankpa4-187-158	50.74	15.06	52.29	16.91	11.94	3.08	20.43	7.77
S19/Ankpa4-188-159	57.25	20.17	51.09	33.53	12.14	5.36	20.86	5.57
S19/Ankpa4-190-160	34.86	15.46	57.98	10.49	2.10	4.75	22.88	8.84
S19/Ankpa4-191-161	50.25	14.17	53.09	0.00	0.00	4.76	27.86	7.59
S19/Ankpa4-192-162	58.25	17.17	52.09	9.53	4.14	2.26	19.86	6.59
S19/Ankpa4-193-163	60.74	21.06	73.29	34.91	11.24	2.98	23.43	12.77
S19/Ankpa4-194-164	50.79	21.53	76.29	16.60	6.02	4.18	27.25	14.39
S19/Ankpa4-195-165	25.74	14.49	57.47	39.51	12.53	4.43	22.56	10.16
S19/Ankpa4-196-166	35.74	12.49	51.47	27.51	6.53	2.73	18.56	17.16
S19/Ankpa4-197-167	59.79	20.53	74.29	110.60	27.82	2.38	26.25	14.39
S19/Ankpa4-198-168	28.74	21.06	74.29	12.90	14.94	2.88	26.43	16.77
S19/Ankpa4-199-169	37.77	18.51	66.38	58.06	15.18	3.96	24.40	12.27
S19/Ankpa4-20-18	24.25	14.17	53.09	37.53	8.34	4.46	24.86	6.59
S19/Ankpa4-200-170	35.74	15.49	57.47	45.51	16.53	4.33	20.56	11.16
S19/Ankpa4-202-171	33.48	16.76	60.86	15.44	10.37	3.64	23.90	12.09
S19/Ankpa4-203-172	48.74	18.06	67.29	6.91	1.94	3.68	23.43	11.77
S19/Ankpa4-204-173	58.74	19.06	69.29	6.91	1.94	4.98	25.43	15.77
S19/Ankpa4-205-174	26.74	13.49	54.47	29.51	8.53	4.03	21.56	9.16
S19/Ankpa4-206-175	46.76	22.06	80.29	52.91	13.44	1.78	29.43	20.77
S19/Ankpa4-207-176	30.79	15.53	16.29	18.60	10.32	4.18	21.25	8.39
S19/Ankpa4-208-177	25.74	16.49	15.47	41.51	13.53	4.03	23.56	11.16

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-21-19	59.48	16.76	62.86	55.44	14.87	3.54	24.90	11.09
S19/Ankpa4-210-178	40.25	15.17	55.09	9.53	4.14	4.36	21.86	10.59
S19/Ankpa4-211-179	39.74	13.06	52.29	8.91	3.94	2.88	20.43	7.77
S19/Ankpa4-212-180	40.74	19.06	69.29	42.91	13.94	4.98	25.43	14.77
S19/Ankpa4-213-181	41.74	20.49	72.47	29.51	8.53	2.63	26.56	12.16
S19/Ankpa4-215-182	28.25	15.17	55.09	57.53	9.24	1.36	21.86	9.59
S19/Ankpa4-216-183	52.48	19.76	79.86	5.44	3.37	0.64	29.9	23.09
S19/Ankpa4-217-184	42.74	17.06	60.29	18.91	5.94	4.18	23.43	12.77
S19/Ankpa4-219-185	26.25	22.17	78.09	5.53	7.84	4.46	24.86	16.59
S19/Ankpa4-220-186	32.25	20.17	70.09	0.00	0.00	3.16	25.86	15.59
S19/Ankpa4-222-187	21.79	21.53	78.29	34.60	8.82	5.08	27.25	14.39
S19/Ankpa4-223-188	35.48	16.76	59.86	7.44	6.37	3.04	23.09	12.90
S19/Ankpa4-224-189	35.74	13.06	53.29	44.91	20.94	3.18	27.43	10.77
S19/Ankpa4-226-191	55.25	17.17	59.09	61.53	14.34	1.76	22.86	10.59
S19/Ankpa4-228-192	28.25	16.17	57.09	17.53	6.84	4.96	22.86	12.59
S19/Ankpa4-23-20	32.48	19.76	69.86	15.44	10.37	3.34	25.90	16.09
S19/Ankpa4-231-191	25.48	12.76	50.86	47.44	10.17	4.14	19.90	8.09
S19/Ankpa4-232-194	50.25	17.70	59.09	47.53	7.24	4.16	22.84	11.59
S19/Ankpa4-232-195	30.48	11.76	48.86	23.44	9.37	5.04	17.90	6.09
S19/Ankpa4-234-196	26.25	22.17	78.09	21.53	8.14	2.16	29.86	22.59
S19/Ankpa4-234-197	29.79	21.53	78.29	96.60	19.32	2.08	27.25	19.39
S19/Ankpa4-235-198	52.25	13.17	62.09	57.53	13.34	1.76	23.86	12.59
S19/Ankpa4-237-200	53.74	15.49	57.47	31.51	10.53	4.23	22.56	8.61
S19/Ankpa4-238-201	29.74	22.49	78.47	56.51	14.03	2.13	27.56	18.16
S19/Ankpa4-239-202	26.74	12.49	48.47	31.51	8.53	4.93	17.56	7.16

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-241-203	47.74	22.06	78.29	62.91	15.94	4.88	29.43	21.77
S19/Ankpa4-242-204	32.74	15.49	63.47	49.51	28.53	2.33	24.56	12.16
S19/Ankpa4-243-205	33.25	16.17	56.09	3.53	2.14	4.66	19.86	10.59
S19/Ankpa4-244-206	39.74	22.06	79.29	14.91	9.94	3.88	30.43	22.77
S19/Ankpa4-245-207	43.74	14.06	53.29	8.91	3.94	5.08	20.43	8.77
S19/Ankpa4-247-208	49.43	16.76	59.86	25.44	10.07	4.04	22.90	11.09
S19/Ankpa4-248-209	39.79	16.53	61.29	0.00	1.32	3.78	23.25	12.39
S19/Ankpa4-249-210	64.48	14.76	56.84	45.44	9.77	4.07	22.90	11.09
S19/Ankpa4-25-21	39.48	11.76	49.86	1.44	3.37	2.24	23.90	7.09
S19/Ankpa4-251-211	32.74	17.49	61.47	43.51	14.53	4.83	23.56	7.16
S19/Ankpa4-252-212	48.25	17.17	59.09	0.00	0.84	4.16	23.86	12.59
S19/Ankpa4-253-213	45.25	18.17	68.09	25.53	9.54	4.16	24.86	13.59
S19/Ankpa4-254-215	24.79	21.53	77.29	132.6	26.52	1.08	26.25	15.39
S19/Ankpa4-255-215	35.74	19.49	52.47	27.51	6.53	4.73	20.56	8.16
S19/Ankpa4-26-22	41.25	20.17	69.09	37.53	8.34	3.26	24.86	13.39
S19/Ankpa4-27-23	48.78	15.49	56.47	31.51	8.53	4.33	22.56	9.16
S19/Ankpa4-28-24	47.25	21.17	74.09	43.53	9.84	2.66	25.86	14.59
S19/Ankpa4-29-25	56.74	19.49	69.47	47.51	16.53	3.03	25.56	14.16
S19/Ankpa4-3-2	41.74	21.49	78.47	27.51	6.53	3.03	25.56	14.16
S19/Ankpa4-30-26	45.74	13.49	53.47	27.51	6.53	4.63	20.56	7.16
S19/Ankpa4-31-27	59.79	16.53	6.29	48.60	12.32	3.98	23.25	9.39
S19/Ankpa4-32-28	41.48	21.76	77.86	23.44	6.87	2.04	77.90	19.09
S19/Ankpa4-33-29	48.74	18.96	66.29	16.91	11.94	1.08	23.43	12.77
S19/Ankpa4-339-266	47.25	17.17	67.09	75.53	17.84	1.96	22.86	10.59
S19/Ankpa4-34-30	28.78	22.49	79.47	31.51	10.53	3.53	28.56	19.16

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-36-31	25.74	23.06	82.29	38.91	12.64	1.18	34.43	26.77
S19/Ankpa4-37-32	49.48	12.76	62.86	0.00	0.00	1.84	23.90	18.09
S19/Ankpa4-38-33	24.25	18.17	64.09	0.00	0.00	3.66	23.86	11.50
S19/Ankpa4-39-34	47.79	13.53	53.29	0.60	3.32	3.18	20.25	8.39
S19/Ankpa4-40-35	31.74	22.06	79.29	70.91	14.74	3.78	23.43	19.77
S19/Ankpa4-42-36	49.48	16.76	61.86	29.44	6.57	2.94	23.9	12.09
S19/Ankpa4-43-37	49.52	14.17	54.09	0.00	0.00	4.76	26.86	7.59
S19/Ankpa4-44-38	54.48	12.76	51.86	45.44	12.87	1.94	19.90	8.09
S19/Ankpa4-46-39	42.74	13.49	52.47	27.51	6.53	4.53	20.56	8.16
S19/Ankpa4-47-40	29.48	16.76	61.86	33.44	19.37	4.34	22.90	12.09
S19/Ankpa4-48-41	36.48	19.76	72.86	29.44	17.37	4.94	26.90	18.09
S19/Ankpa4-49-42	42.25	20.17	69.09	17.53	3.34	3.36	24.86	13.59
S19/Ankpa4-5-4	55.48	17.76	64.86	43.44	16.07	3.24	23.90	13.09
S19/Ankpa4-50-43	52.25	20.17	73.09	91.53	16.04	1.16	24.86	13.59
S19/Ankpa4-51-44	48.74	20.06	73.29	44.91	14.64	3.08	27.43	17.77
S19/Ankpa4-52-45	39.79	20.53	74.29	0.00	0.00	1.68	25.25	12.39
S19/Ankpa4-53-46	38.74	17.49	63.47	31.51	10.53	3.73	24.56	11.16
S19/Ankpa4-55-47	52.74	13.59	53.47	33.51	12.53	1.23	30.56	7.16
S19/Ankpa4-56-48	51.74	16.49	60.47	29.51	8.53	3.93	22.56	11.16
S19/Ankpa4-57-49	42.48	20.76	73.86	57.44	15.37	1.44	25.90	15.09
S19/Ankpa4-58-50	45.74	20.49	72.47	27.51	6.53	2.63	26.56	16.16
S19/Ankpa4-6-5	34.74	16.49	60.47	31.51	10.53	3.93	22.56	10.16
S19/Ankpa4-60-51	31.25	18.17	66.09	31.53	6.84	0.76	23.86	12.59
S19/Ankpa4-61-52	61.74	16.49	60.47	59.51	12.93	4.43	23.56	11.61
S19/Ankpa4-62-53	48.25	20.17	73.09	0.00	0.00	2.66	26.86	10.59

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-63-54	32.74	15.49	57.47	37.51	16.53	1.33	22.56	7.16
S19/Ankpa4-64-55	48.48	16.76	80.46	33.44	9.37	3.64	22.90	11.09
S19/Ankpa4-65-56	27.48	21.76	78.86	53.44	29.37	4.94	28.90	20.09
S19/Ankpa4-66-57	41.74	21.49	79.47	39.51	12.53	2.03	29.56	21.16
S19/Ankpa4-67-58	59.25	21.17	73.09	13.53	5.54	4.26	26.86	17.59
S19/Ankpa4-68-59	45.48	15.76	58.86	0.00	1.37	3.94	22.90	8.09
S19/Ankpa4-69-60	24.74	22.06	80.29	76.91	25.24	2.68	30.43	23.77
S19/Ankpa4-7-6	54.79	15.53	57.29	0.60	3.32	1.88	22.25	9.39
S19/Ankpa4-70-61	39.79	19.53	69.29	12.60	4.62	2.78	24.25	13.39
S19/Ankpa4-72-63	35.25	15.17	56.09	29.53	6.34	2.36	22.86	10.59
S19/Ankpa4-73-64	43.25	20.17	17.09	37.53	13.54	2.46	25.86	14.59
S19/Ankpa4-74-65	30.74	23.49	82.47	41.51	13.53	0.73	30.56	23.16
S19/Ankpa4-76-66	56.79	12.53	51.29	0.00	1.32	1.08	20.25	6.39
S19/Ankpa4-77-67	44.48	13.76	53.86	1.44	2.07	5.44	20.90	7.09
S19/Ankpa4-79-62	43.74	16.49	60.47	31.51	10.53	3.93	23.56	11.16
S19/Ankpa4-79-68	50.48	22.76	80.86	5.44	5.37	1.74	30.90	24.09
S19/Ankpa4-8-7	33.79	19.53	70.29	66.60	16.83	27.78	25.25	14.39
S19/Ankpa4-81-69	57.74	17.06	60.29	18.91	5.94	3.88	23.43	11.77
S19/Ankpa4-82-70	39.74	22.06	79.29	26.91	11.94	4.48	30.43	10.77
S19/Ankpa4-83-71	56.74	15.49	58.47	29.51	8.53	2.53	22.56	10.16
S19/Ankpa4-84-72	26.48	14.76	56.86	0.00	1.37	4.14	22.90	12.09
S19/Ankpa4-85-73	35.48	21.76	78.86	0.00	5.37	2.74	28.90	21.09
S19/Ankpa4-86-74	57.48	14.76	55.86	39.44	8.57	1.94	20.90	15.09
S19/Ankpa4-88-75	49.48	14.76	56.84	25.44	5.77	4.04	21.90	10.09
S19/Ankpa4-89-102	46.79	17.53	76.29	44.60	2.02	0.28	26.25	16.39

TREATMENT	CW	DE	DF	GY	GYP	IL	PH	PL
S19/Ankpa4-89-76	57.79	19.53	69.29	54.60	10.92	1.38	24.25	13.39
S19/Ankpa4-9-8	56.74	15.06	55.29	6.91	1.94	4.78	21.43	7.77
S19/Ankpa4-90-77	42.48	19.76	70.86	69.44	4.07	2.84	25.90	16.09
S19/Ankpa4-91-78	43.74	20.49	72.47	53.51	19.53	2.73	26.56	15.16
S19/Ankpa4-92-79	23.79	18.53	68.29	50.60	17.32	4.18	24.25	14.39
S19/Ankpa4-93-80	25.79	21.53	78.29	48.60	16.62	0.98	29.25	21.39
S19/Ankpa4-94-81	50.48	22.76	81.86	55.44	14.87	3.74	31.90	23.09
S19/Ankpa4-95-82	24.74	20.06	52.29	6.91	1.94	2.18	20.43	7.77
S19/Ankpa4-96-83	39.25	18.17	66.09	13.53	11.84	1.46	22.86	10.59
S19/Ankpa4-96-84	58.48	13.76	55.86	29.44	11.37	4.14	21.19	10.09
S19/Ankpa4-97-85	24.74	15.49	72.47	29.51	8.53	2.63	26.56	15.16
S19/Ankpa4-98-86	40.25	21.17	77.09	3.53	0.00	3.56	21.86	18.56
S19/Anpa4-109-90	24.74	12.06	49.29	6.91	1.94	2.08	22.43	10.77
SONGKHLA	38.00	15.60	56.80	48.40	11.56	4.46	23.20	9.60
TIGD	49.20	14.00	53.80	14.80	6.54	3.24	20.60	8.00
UKZN 1	44.60	15.00	57.00	14.40	4.80	3.72	23.40	11.00
UNISWA	46.80	21.00	72.80	52.80	17.30	2.02	26.80	17.40