

**MOLECULAR, ENVIRONMENTAL AND NUTRITIONAL EVALUATION OF
BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.) FOR FOOD
PRODUCTION IN NAMIBIA**

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc.), an indigenous African legume valued for its drought tolerance, is popular in most parts of Africa. The study aimed to study environment effects on six landraces of Bambara groundnut (Nam 1759/3, Uniswa Red, S-19/3, KFBN 9709, KFBN0105 and KFBN 0116) and to evaluate their molecular and nutritional compositions. The effects of different sowing dates and watering regimes on the growth and development of the landraces were studied. There were two sowing dates; one in the hot season and dry one late in the season during winter season. There were also two watering regimes; one where watering was done twice a week until harvesting and a drought treatment with no irrigation until final harvest. Parameters recorded included germination percentage, water use efficiency and crop yield. Genetic diversity in the 6 landraces of Bambara groundnut was evaluated using Random Amplified Polymorphic DNA (RAPD) and microsatellite markers. Nutritional evaluation was done on the six landraces by doing proximate analysis using the association of official analytical chemists' methods. The study on acceptability, processing and utilization of Bambara groundnut in Namibia was done through a survey using a questionnaire to collect data. The questionnaire was presented to farmers and consumers of Bambara groundnut in the northern part of Namibia, where the crop is mainly consumed.

No significant difference was observed among the different landraces between the two watering regimes as far as growth and development analysis was concerned. Field sown earlier in the year gave a larger harvest (204.396 – 336.535kg/ha) and the field sowed during the winter season doing extremely poor (11.778 – 65.125kg/ha). All landraces showed significant difference among the different sowing dates ($\chi^2= 9.269$, $\chi^2= 9.846$, $\chi^2= 8.000$, $\chi^2= 9.269$, $\chi^2= 9.269$, $\chi^2= 9.302$). Looking at their yield at harvest, the landraces were classified based on BAMnut Model – Crop Biomass and Pods Model and they ranged from unsuitable (Uniswa Red) to moderately suitable (KFBN 0105). Calculated water use efficiency showed that in winter the plant produced more per kg water than the ones in hot seasons. Strangely enough, some landraces produced more yield per kg water in non-irrigated plots than irrigated.

RAPDs revealed high levels of polymorphism among the landraces. Polymorphism ranged from 57% to 100% with an average of 85% for the 7 RAPD primers evaluated, while microsatellites gave low levels of polymorphism ranging from 0% to 100%, with average polymorphism of 29%. All landraces were shown to be closely similar with the lowest similarity at about 88% similarity, and highest similarity of 98% between Nam 1759/3 and KFBN 9709. The low Shannon indices (0.426 – RAPDs and 0.093 – microsatellites) indicated a low genetic diversity among the six landraces

The results obtained for nutritional evaluation of the six landraces, were consistent with previous studies on Bambara groundnut seeds. Moisture content ranged between 7.3% and 7.8%, ash content from 3.4% and 4.1%, fat content 2.1% and 3.9%, protein content 18.2% and 22.2%, and carbohydrates between 63.0% and 66.6%. There was no significant difference for carbohydrates, fats and protein contents among the different landraces as far as nutrient content was concerned among the different landraces ($\chi^2= 2.720$, $\chi^2= 8.440$, $\chi^2= 3.090$, $\chi^2= 8.520$, $\chi^2= 6.440$, $\chi^2= 6.293$) and between the irrigated and non-irrigated plots ($Z = -1.993$, $Z = -1.964$, $Z = -1.964$, $Z = -1.964$, $Z = -1.964$, $Z = -1.964$).

Survey showed that, Bambara groundnut is mostly grown for home consumption and for sale on a small scale. Farmers find that the biggest constraint on the production of Bambara groundnut is the variation in rainfall within the growing season of the crop (heavy rainfall for

the past two sowing seasons). Ninety nine percent of the activities involved during the Bambara groundnut processing are equally divided within the genders and they are done in traditional ways (from field preparation, through storage of seeds or pods to cooking). Bambara groundnut seeds in Namibia are eaten boiled only (either fresh or dry) and farmers as well as consumers in that region are not aware of the different products that can be processed from the seeds.

It was concluded that growth and development of Bambara groundnut is highly affected by cold weather as well as high rain fall. The crop is highly adapted and has a water conserving mechanism, which should be investigated further. Genetically, the landraces are closely related and do not show high diversity. The crop is nutritionally good and can be viewed as a balanced diet crop and can contribute to food security under good agronomic methods. An extensive study comparing wild forms of Bambara groundnut and the known landraces should be done to evaluate their suitability to be domesticated.

Landraces 4, 5 and 6 (KFBN 9079, KFBN 0105 and KFBN 0116 respectively) did well in both hot season and cold season, they can be considered as the ones best suited for the Namibian climate. Base on the outputs from BAM nut Model – Crop Biomass and pods Models in Azam-Ali et.al, 2001, these three landraces can be classified from suitable to moderately suitable based on their yields.

DEDICATION

This thesis is dedicated to the greatest and wisest man on earth, my father, Prof P.B. Ndengejeho; you are the best father anyone can ever have. For all your wisdom and encouragement during this time, may God Almighty bless you, keep you safe and grant you many years with us. I love you dad.

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DECLARATIONS

I, Celine Mukakalisa, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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ACRONYMS

IITA	International Institute of Tropical Agriculture
DNA	Deoxyribose Nucleic Acid
RFLP	Restriction Fragment Length Polymorphism
RAPD	Randomly Amplified Polymorphic DNA
AFLP	Amplified Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
LR	Landrace
DAS	Days After Sowing
EC	Emergence Count
AOAC	Association of Official Analytical Chemists
GA	Growth Analysis
WUE	Water Use Efficiency
SE	Standard Error
BAM nut	Bambara groundnut

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CHAPTER 1

1. INTRODUCTION

1.1. General introduction

In semi-arid Africa, where rainfall is low and soil fertility is poor, crop production conditions are difficult to control. Increased food production requires that crops and crop genotypes be well adapted to local environments. An important aspect of plant adaptation is the way reproductive development is influenced by environmental factors. Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is one of the crops that could be used to increase food production. It is a leguminous food crop that is already widely cultivated in tropical Africa, with a reported high drought tolerance (Brink, Sibuy, Tarimo and Ramolemana, 2000).

Although Bambara groundnut is an important crop for semi-arid Africa, yields are very low, typically 300 – 800 kg ha⁻¹. The low yield can be attributed to many constraints, such as the amount and distribution of rainfall, high or low temperatures, poor soils, diseases and pests, inappropriate agronomic practices and lack of improved cultivars (EU Project number TS3*CT920121, 1996).

Bambara groundnut has a large number of landraces throughout Africa where growers have preserved its genetic diversity without serious attempts by scientists to exploit or improve particular landraces (Massawe, Dickson, Roberts and Azam-Ali, 2002). As awareness of the potential of under-utilised crops like Bambara groundnut increases, both in terms of increasing food production and improving crop diversity, there is a need to study and exploit

these crops to improve their productivity (Massawe, Dickinson, Roberts and Azam-Ali, 2002).

Previous studies (as mentioned in BAMLINK ANNEX, 2004) were done on the crop under controlled conditions where it was revealed that there is quite a lot of diversity within the Bambara groundnut population and it also showed that the crop is very tolerant to high temperatures. Results from field experiments in Africa and controlled environments in Europe, were incorporated into a computer model of Bambara groundnut to predict yields under rainfed and well-watered conditions. Model predictions indicated that yields of 4.5 t ha^{-1} were possible for existing landraces in suitable agro-ecological regions and with appropriate management. In controlled environments, pod yields of 3.5 t ha^{-1} were obtained and rainfed yields of 3.0 t ha^{-1} were achieved in African field conditions (BAMLINK ANNEX, 2004). Grain yield of Bambara groundnut has been reported to be different from one country to the other: Chad and Cameroun 800 kg ha^{-1} , Benin $450\text{-}650 \text{ kg ha}^{-1}$, Congo $350\text{-}650 \text{ kg ha}^{-1}$ and Ghana $714\text{-}1100 \text{ kg ha}^{-1}$ (Ellah and Singh, 2008). Begmann (1987) in Ellah and Singh (2008), stated that the yield potential of Bambara groundnut in Nigeria is higher than any African country, the yield ranges from $500\text{-}2600 \text{ kg ha}^{-1}$. Studies also demonstrated that, in addition to its significant yield potential in irrigated conditions, Bambara groundnut is more drought-tolerant than other legumes such as peanut (*Arachis hypogaea*) (BAMLINK ANNEX, 2004). These studies concluded that there is considerable potential to improve the performance of existing Bambara groundnut landraces even in hostile environments where yields are typically less than 300 kg ha^{-1} (BAMLINK ANNEX 2004).

1.2. Statement of problem

Africa is one of the continents faced with problems that arise from malnutrition and famine. Semi-arid Africa has conditions (i.e. high temperatures and low rainfall) that are not favored by many crops to sustain the growing human population. Most countries that are found in famine ravaged regions depend on food donated from United Nations (UN) or other organization for survival. At the regional level, Sub-Saharan Africa had the highest proportion of undernourished and malnourished from 1997-1999 at 34%. Asia and Pacific (excluding China) follows at 20%, the Latin American and the Caribbean with 11%, and the Near East and North Africa region at 9% (www.worldhunger.org/articles/global/ray.htm). According to the Food and Agricultural Organization (FAO) of the United Nations, 850 million people worldwide were malnourished in 1999-2005 and the number of malnourished people has recently been increasing. According to Jean Ziegler (the UN special rapporteur on the Right to Food for 2002 to March 2008), mortality due to malnutrition accounted for 58% of the total mortality in 2006 ([http://en.wikipedia.org/wiki /Malnutrition](http://en.wikipedia.org/wiki/Malnutrition)).

Bambara groundnut has the potential to contribute to the food security of resource- and nutritionally-poor communities throughout the semi-arid tropics. This research seeks to identify characteristics of Bambara groundnut genotypes that enhance productivity, nutritional quality and tolerance to environmental stresses so that they may contribute significantly to food security, agricultural diversification and income generation.

Namibia, being a predominantly arid country has to be vigilant in food production to feed its population. The country's climate is unpredictable, crop failure and livestock mortalities are usually the consequence. Despite all the efforts being made, the unpredictable weather continues to take its toll on the agricultural sector and Namibia like its Southern African

neighbours, has been facing food crisis as a result of poor 2001-2002 rain season (<http://www.fao.org/docrep/005/Y4172M/er2/namibia.htm>). Therefore, it is important for the Namibian government to find ways to improve food production starting with crops that are adapted to its environment, and that contain required dietary constituents to avoid starvation and loss of human life.

This project will enable the evaluation of Bambara groundnut as a stable food source that can be produced on large scale to feed the entire population.

1.3. Objectives and hypotheses

1.3.1. Objectives

The specific research objectives of the study are to:

- a) Compare the productiivity of six different landraces of Bambara groundnut commonly used by farmers grown in two different seasons (summer (December – March) for heat stress (D₁) and winter (April – July) for cold stress (D₂).
- b) Compare the productivity of the six different landraces to moisture stress
- c) Determine if there is any genetic diversity among the six landraces
- d) Evaluate and compare the nutrient content of the seeds of six different landraces
- e) Survey acceptability of products from Bambara groundnut by end-users and assess potential for increased demand and utilization of Bambara groundnut in Namibia.

1.3.2. Hypotheses

- a) The seeds sown in summer will have higher production (yield) than the ones sown in winter, since Bambara groundnut is a tolerant crop and very adaptable to hot temperatures, while low temperatures cause late on setting of flowers as well as pods production.

- b) Irrigated plants will have higher yield than non-irrigated plants, since applying water assures sufficient soil moisture is available for good plant growth and development.
- c) The genetic makeup of the six landraces is sufficiently different to elicit different phenotypes.
- d) The different landraces will have different levels of nutrients in their seeds, since they exhibit different phenotypical traits.
- e) Human preference of Bambara groundnut products will depend on the type of seeds/landrace used, since end products will have different taste and preparation may vary from one type to another.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Ecology, importance and uses of Bambara groundnut

Bambara groundnut (*Vigna subterranean* (L.) Verdc.) is an indigenous African legume that has been cultivated in Africa for centuries (Ellah and Singh, 2008). Bambara groundnut has for many centuries been cultivated in the tropical regions of Africa south of the Sahara where it is indigenous. Major producers are Nigeria, Niger, Ghana and Ivory Coast, but it is widely grown in eastern Africa and Madagascar (Lineman and Azam-Ali, 1993). The centre of origin of Bambara groundnut is Africa, in Madagascar. The wild forms are found in Jos, Yola, Ogoja in cross River State (Ellah and Singh, 2008).

Wringley (1981) in Barey (2000) states that Bambara groundnut grows best in soils of 5.0-6.5 pH with 600-1200 mm annual rainfall. He also states that it is very adaptable to hot temperatures and can tolerate low rainfall. Bambara groundnut displays a range of traits conferring drought resistance and is a crop especially suited to low input farming (Massawe, Roberts, Azam-Ali and Deavey, 2001). However, the crop is characterised by variable and unpredictable yields for reasons that have not yet been identified (Massawe, Dickinson, Roberts and Azam-Ali, 2002).

Important attributes of Bambara groundnut include: tolerance to drought, high nutritive value, adaptation to marginal conditions and relative resistance to pests and diseases (Ntundu, Shillah, Marandu and Christiansen, 2004). Bambara groundnut provides a rich source of protein and, along with other local sources of protein, plays a major nutritional and socio-economic role in the semi-arid regions of Africa (Massawe, Azam-Ali and Roberts, 2003). It is a legume grown mainly by women for sustenance of their families (Mwale, Azam-Ali and Massawe, 2005). It is ranked third most important grain legume after peanut (*Arachis hypogea* (L.)) and cowpea (*Vigna unguiculata*) (Barey, 2000). The seed is regarded as a completely balanced food because it is rich in iron 4.9-48 mg/100g, protein 18.0-24.0%, ash 3.0-5.0%, fat 5.0-7.0%, fibre 5.0-12.0%, potassium 1144-1935mg/100g, carbohydrates 51-70%, oil 6-12% and energy 367-414kcal/100mg (Adu-Dapaah and Sangwa, 2004).

Bambara groundnut is predominantly grown for human consumption. Immature seeds are consumed fresh or grilled. Seeds can be boiled in the immature green state, either shelled or unshelled, until quite soft (Lineman and Azam-Ali, 1993). In many countries in West Africa, fresh pods are boiled with salt and pepper and eaten as a snack. In East Africa, the beans are roasted pulsed and used in preparing soup (Massawe, Mwale, Azam-Ali and Roberts, 2005). Bambara groundnut seeds can be used to produce vegetable milk that is comparable to soy milk. Protein functionality test on the seeds indicate that it can compete with or replace other conventional flour in a range of processed products. Bambara groundnut can also be used as feed to pigs and poultry and haulm as fodder (Lineman and Azam-Ali, 1993). As a leguminous crop, Bambara groundnut is useful in crop rotation as it acts as a source of residue nitrogen for the subsequent crop through nitrogen fixation (Mukumbira, 1985, cited by Ntundu, Shillah, Marandu and Christiansen, 2004). Apart from being consumed at home,

in Zimbabwe, it is now canned at commercial level. In Zimbabwe, it is reported that traders from around the country and across the borders were increasingly buying the crop at Ngundu market at an average price of US\$ 0.50/kg (Chbudu, 1995, cited by Makanda, Tongoona, Madamba, Icishahayo and Derera, 2009) and up to US\$0.30 in South Africa (Swanevel, 1998, in Makanda, Tongoona, Madamba, Icishahayo and Derera, 2009). The crop is, therefore, becoming an important economic product (Makanda, Tongoona, Madamba, Icishahayo and Derera, 2009).

2.2. Abiotic factors influencing Bambara groundnut

The production and consumption of Bambara groundnut is mostly restricted to the semi-arid regions of Africa where rainfall is erratic and low and losses to run-off, drainage and evaporation may leave only a small proportion available for crop growth. Lack of moisture (drought) is therefore a common occurrence for rainfed crops in these regions during some or all of their growth (Massawe, Mwale, Azam-Ali and Roberts, 2005).

Water is one of the most important environmental factors for plant growth and development. Water deficiency will restrict the whole growing process of the plants including external and internal structure and metabolism. However studies showed that moderate water deficiency could enhance plant growth (Diam, Li, Wang and Ji, 2002). Water deficit causes advanced or delayed flowering depending on the species. In severe cases, water deficit cause reproductive failure. The most relevant physiological measure of plant water deficit is relative water content (RWC), which may be used as an indicator of drought susceptibility or tolerance (Blum, 1997).

Temperature and photoperiod are the main factors influencing development of annual crops and there are often large genotypic differences in the response to these factors (Brink, Sibum, Tarimo and Ramolemana, 2000). In many Bambara groundnut selections, the onset of flowering is photoperiod-insensitive, but the onset of pod growth ('podding') is retarded by long photoperiods (Brink, Sibum, Tarimo and Ramolemana, 2000).

Canopy development is important and is mainly driven by temperature. The crop exhibits a considerable degree of phenotypic diversity in morphology and growth habit. It is therefore likely that the influence of temperature on vegetative development is not uniform among genotypes (Massawe, Dickinson, Roberts and Azam-Ali, 2002). However, the plant grows better in bright sunshine and at average temperatures of 20 – 28°C (Lineman and Azam-Ali, 1993).

Bambara groundnut genotypes have been shown to exhibit variable responses to different planting dates (Makanda, Tongoona, Madamba, Icishahayo and Derera, 2009). Germination of Bambara groundnuts increases from 16.8° until 32.5°C, where it reaches a peak and declines until 39.5°C. It usually takes seven to 15 days under favourable temperature (28.5 to 32.5°C) for Bambara groundnut to germinate; but under lower temperatures, it takes up to 31 days with some seeds remaining dormant indefinitely. Soil temperature is critical at the time of planting to ensure optimum germination and plant establishment. This has a bearing on early planting in Southern Africa where winter temperatures are low. As a result, germination is delayed and results in poor plant stand (Makanda, Tongoona, Madamba, Icishahayo and Derera, 2009).

2.3. Genetic studies on Bambara groundnut

Bambara groundnut has not been improved through coordinated breeding programmes and therefore different genotypes of this crop still exist as landraces. By description, considerable genetic variation exists within and between landraces for review of landraces; and hence, landraces are often well adapted to a wide range of environmental conditions (Massawe, Dickinson, Roberts and Azam-Ali, 2002). The major germplasm collection of Bambara groundnut is held by the International Institute of Tropical Agriculture (IITA) in Nigeria. Although a number of scientists have collected Bambara groundnut landraces from different parts of Africa and beyond, these valuable genetic resources have not been fully exploited (Massawe, Mwale, Azam-Ali and Roberts, 2005).

The detection and exploitation of naturally occurring variation at the DNA level in many organisms has been made possible by the advent of molecular markers. Molecular techniques such as Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) provide powerful tools for the study of genetic diversity (Massawe, Dickinson, Roberts and Azam-Ali, 2002). Experience gained from these techniques in major crops provides an opportunity to apply similar approaches to Bambara groundnut and hence accelerate the acquisition of knowledge for a crop that is cultivated principally by marginal farmers in semi-arid Africa (Massawe, Dickinson, Roberts and Azam-Ali, 2002).

Relatively few genetic studies have been conducted on the population structure of *V. subterranea* and genetic diversity among cultivates. A study done using isozyme analysis found high genetic similarities between wild and domesticated Bambara groundnut

accessions and concluded that the wild Bambara groundnut is the progenitor of the domesticated form, both being characterized by low genetic diversity (Amadou, Bebeli and Katlisikes, 2001). Although isozyme analysis and RFLPs are a source of readily obtainable genetic information which is easily reproduced, they often do not show polymorphisms which are necessary to determine variation within a group of genetically similar individuals (Baillie, Higgins, Kerestes, McGrew, Richards, Richards and Salb, 2009). RAPD markers provide a useful tool to identify and estimate genetic diversity among Bambara groundnut landraces. This technique is simple, rapid, requires small amounts of DNA and does not involve radioisotopes (Massawe Dickinson, Roberts and Azam-Ali, 2002). The RAPD procedure is technically the simplest variation of arbitrarily primed Polymerase Chain Reaction (PCR) methods. Primers with ten nucleotides and a GC content of at least 50% are generally used. The amplification products are separated on an agarose gel and detected by staining with ethidium bromide (Weising, Nyborn, Wolff and Meyer, 1994).

PCR allows the amplification of any DNA sequence of interest to high copy numbers thereby circumventing the need of molecular cloning (Weising, Nyborn, Wolff and Meyer, 1994). The PCR amplification takes place in cycles. In the first cycle, the DNA is heated to separate the strands and then cooled in the presence of a vast excess of primers. The elongation of the primers produces double-stranded molecules. The second cycle of PCR is similar to the first but, after the second cycle; there are four copies of each original molecule. The cycle is repeated from 20 to 30 times, each resulting in a doubling of the number of molecules (Hart and Clark, 1997). This amplification is carried out by the DNA polymerase I enzyme from *Thermus aquaticus*, a bacterium that lives in hot springs. This enzyme is thermostable, meaning that it is resistant to denaturation by heat treatment. On cooling more primers anneal at their respective position (including positions on the newly synthesized strands), and Taq

polymerase, unaffected by the heat treatment, carries out a second round of DNA synthesis (Brown, 1998).

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Overview

The field experiment was conducted in the northern part of the country, at Omahenene Research station, 22°00'00" s, 17°00'00"E, where temperatures range from 6 to 18°C (for minimum temperatures) and 21 to 36°C (for maximum temperatures) and rainfall is low (79 – 365 mm per year). Six different landraces were used for this study. These are landraces that are commonly used by local farmers. Seeds were collected from different research stations and institutes, and had different morphological appearance, such as seeds size and color differences. Most of these landraces originated from Namibia while others are believed to have originated from neighboring countries (see *Table 1*).

Sowing of the seeds was done into two seasons, during the dry and hot season, to test for the tolerance of the crop to heat stress and the cold season to test for the tolerance of the crop to cold stress. The two seasons were chosen so that a suited season for the crop's growth and development can be identified. The tolerance of drought stress (low moisture) was studied by dividing the field into two sections, irrigated and non-irrigated (see *Figure 2*).

Laboratory work was divided into two, the nutritional study and the genetic diversity study. Nutritional analysis was done using analytical methods and in triplicates. Proximate analysis of the seeds which includes moisture, protein, fat, ash and carbohydrate content was determined by standard methods of Association of Official Analytical Chemists (AOAC).

Genetic diversity study of the crop was done using two types of molecular markers, RAPDs and microsatellites.

Table 1: The six landraces of Bambara groundnut and their origin

N ^o	Landrace name	Origin	Testa colour
1	Nam179/3	Namibia	Brown
2	Uniswa Red	Swaziland	Red
3	S-19/3	South Africa	Black
4	KFBN9709	Namibia	Brown
5	KFBN0105	Namibia	Dark brown with dots
6	KFBN0116	Namibia	Yellow with brown patches

Genomic DNA (gDNA) was isolated using C.E.Z.N.A.TM Plant DNA Mini Kit short protocol from OMEGA, the DNA extract was then analyzed to assess the quality and yield. The RAPD primers used were stated in previous studies for Bambara groundnut study and were able to work for the study, while the microsatellite primers used were produced specifically for marama bean and were used to verify if they can work as both plant species are both legumes. *Figure 1* shows a diagrammatic representation of overall methodology used in the study.

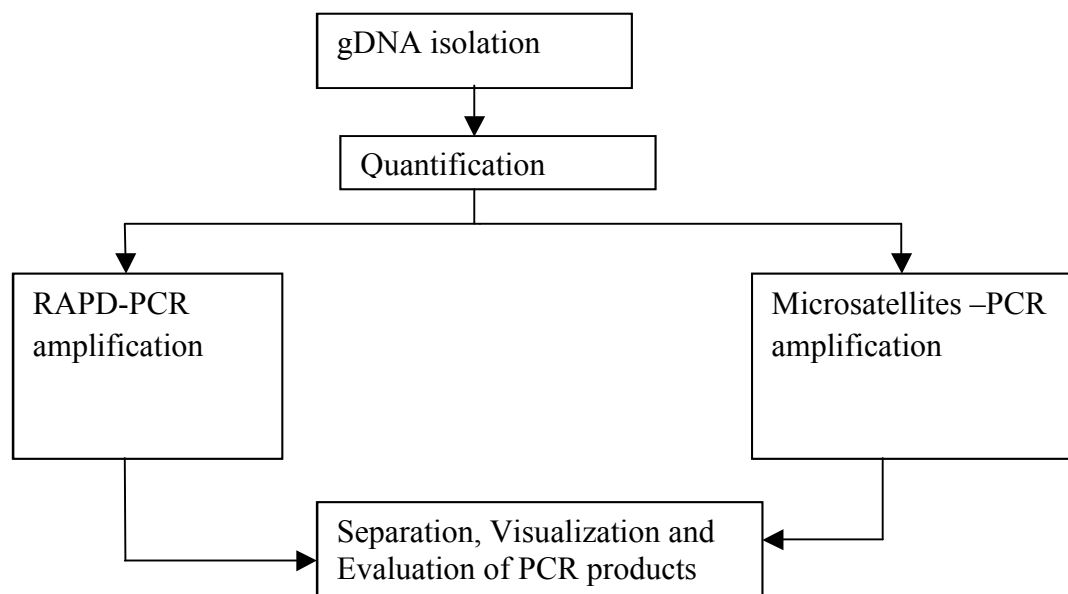


Figure 1: Overview of methodology to assess genetic diversity in Bambara groundnut

Bambara groundnut in Namibia is mostly eaten by people in the northern part of the country; therefore the survey to test for acceptability of the crop’s products and assess its potential and utilization was done there. The region was chosen based on advice given by the regional agricultural extension officer. Questionnaires were administered to farmers in an interview format at the selected villages.

3.2. Field work

3.2.1. Field preparation

A field was chosen in the Northern part of Namibia (at Omahenene Research Station) where the climate conditions are suitable for Bambara groundnut growth. The layout of the field was in such a way that each sowing date was divided into four replications, and had irrigated and non-irrigated areas (see *figure 2*).

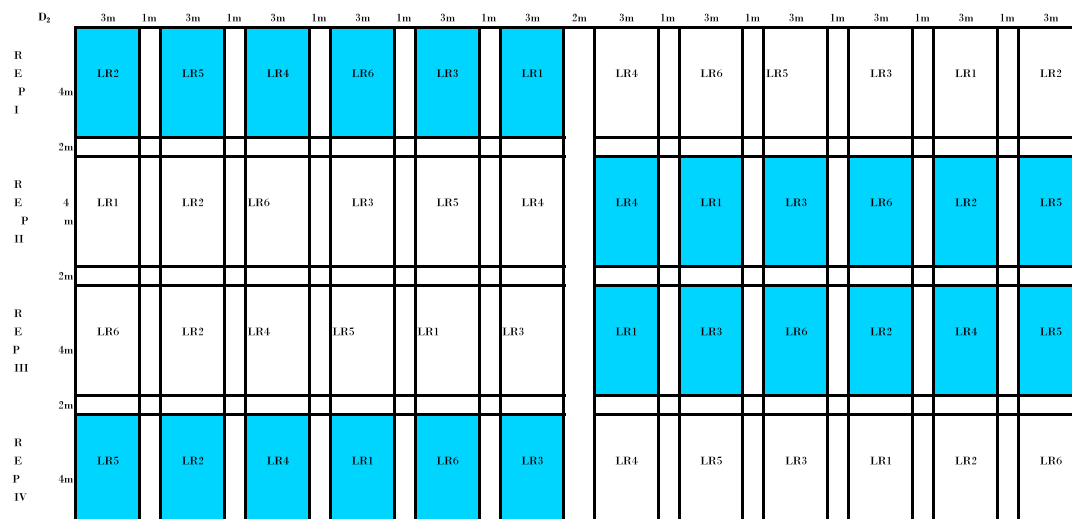


Figure 2: Field design. LR₁ to LR₆ indicate the six landraces, while REP I to REP IV stand for replication 1 to 4. Shaded areas represent irrigated plots.

The experiment was arranged as a factorial experiment, using a split plot lay out in a complete randomized block design. Each box represents a plot of 4m x 3m and each row represents a block (i.e. replicate). The irrigated section of the field (shaded) was irrigated every second week. 10 litres of water (2x5L watering cans) were used for each plot.

3.2.2. Sowing of seeds

Seeds were sown on two different sowing dates. The first sowing date (D_1) was on 21st December, when temperatures are high, for the heat stress study. The second sowing date (D_2) was on 4th June, when temperatures have dropped, the cold stress study. In one subplot there was 32 stations and in each station one seed was sown, therefore, about 32 plants were expected to emerge in the subplot.

3.2.3. Data collection

Emergence count was done daily from the time the first seedling appeared above ground (7 days after sowing), until 21 days after sowing (DAS), when all seeds were expected to have germinated. Additional information was collected, namely, days to flowering and days to podding.

The plant's water status was described in terms of leaf Relative water content (RWC), which can be used as indicator of drought susceptibility or tolerance (Collinson, Clawson, Azam-Ali and Black, 1996). RWC was calculated using the Barrs and Weatherley method (1962) in Collinson, Clawson, Azam-Ali and Black, 1996. The formula used is as follows: $RWC = [(FM-DM) / (TM-DM)] \times 100$, where FM is the fresh mass of 30 leaves, DM is the dry mass

of the leaves, and TM is the turgo mass of the leaves. TM was obtained by soaking the leaves in water for 8 hours. DM was obtained by placing the sample in the oven at 80°C for 24 hours.

Water use efficiency (WUE) of the crop which describes the plant's productivity (Tate, 2000), for each landrace was calculated as above ground biomass (BIO) (i.e. mass of leaves, petioles and stems) divided by seasonal evaporation (ET), a formula described by Songsri, *et al.*, 2009

$$WUE = \frac{BIO (g)}{ET (kg)}$$

$ET = I + (M_i - M_f) - D - R$, where I is the irrigation applications, M_i is the starting soil moisture before sowing, M_f is the soil moisture at final harvest, D is the soil drainage, and R is the surface run off. D and R were ignored due to lack of instrumentation, but obtained results were useful in comparing the WUE of the different landraces.

The crop's yield was calculated based on numerous formulae obtained from Professor Sesay at the Botswana College of Agriculture. For individual plant yield, four plants were harvested in selected plots and the average mass used for analysis. Average mass of seeds and /or pods from plots not touched for individual plant yield (3 plots per landrace) were used to calculate total crop's yield (i.e. seed yield (kg/ha) at 10% moisture).

Calculations for yields were done using the following formulae:

Shelling percentage:

$$\frac{\text{Mass of seeds (from 200 pods)} \times 100}{\text{Mass of 200 pods}}$$

Mass of shelled seeds:

$$\frac{\text{Shelling percentage} \times \text{weight of unshelled pods}}{100}$$

Yield at 10% moisture content:

$$\frac{100\text{-seeds weight (air-dried)} - 100\text{-seeds dry weight (oven dried)} \times 100}{100\text{-seeds weight (air-dried)}}$$

Seed yield (kg/ha) at 10% moisture content:

$$\frac{\text{Dry matter (at weighing)} \times \text{mass of shelled seeds} \times 10 \text{ (constant)}}{90 \text{ (constant)} \times \text{area of net plot}}$$

DM (dry matter) at weighing = 100% - moisture content at 10%

Harvest index (HI):

$$\frac{\text{Pods dry mass}}{\text{Plant dry mass (including pods)}} \times 100$$

3.3. Laboratory work

3.3.1. Genetic diversity of the six landraces

3.3.1.1. DNA extraction

Seeds from harvest were sown in the growth chamber in order to use fresh leaves for DNA extraction.

Fresh leaf materials were harvested from the growth chamber and leaf tissue was ground in liquid nitrogen, then 40mg of the material was transferred into clean microfuge tube. 600µl of buffer P1 and 4µl of RNase was added and incubated at 65°C for 10 minutes. The sample was mixed twice during incubation by inverting the tube. 140µl of buffer P2 was added and then vortexed to mix. The tube was then centrifuged at 10,000 x g for 10 minutes. 600µl of supernatant was carefully aspirated into a new microfuge tube making sure not to disturb the pellet or transferring any debris. 300µl of buffer P3 and 600µl of absolute ethanol were then

added and vortexed thoroughly to obtain a homogeneous mixture. 800µl of mixture was applied to a HiBind® DNA column assembled in a 2mL collection tube then centrifuged at 10,000 x g for 1minute. The flow-through liquid was discarded and the collection tube reused. The remaining of the mixture solution was added to the column and centrifuged again as before, then both the collection tube and flow-through liquid were discarded. The column was placed in a second 2mL collection tube and 650µl of wash buffer diluted in absolute ethanol was added, this was then centrifuged at 10,000 x g for 1minute, the flow-through liquid was discarded and the step was repeated with additional 650µl of the wash buffer. After the flow-through liquid was discarded, the empty column was centrifuged for 2minutes to remove residual ethanol and to dry. The column was transferred to a 1.5mL tube and 100µl of elution buffere pre-warmed at 65°C was added and incubated at room temperature for 5 minutes. The tube was centrifuged at 10,000 x g for 1minute to elute DNA. This step was then repeated using a new 1.5mL tube.

3.3.1.2. DNA quantification

After isolation of DNA, quantification and analysis of quality are necessary to ascertain the approximate quality of DNA obtained. The most commonly used methodologies for quantifying the amount of nucleic acid are gel electrophoresis and spectrophotometric analysis. If the sample amount is less, the former is usually preferred (Chimwamurombe, pers comm., 2010).

For this study DNA was quantified using spectrophotometric protocol described by Naomab (2004) and electrophoretic method described by Hoisington, Khairallah and Gonzalez-de-Leon (1994).

a. Spectrophotometric quantification

Bambara groundnut gDNA extracts were diluted in a ratio of 1:100 with TE buffer in a microcuvette and the optical density (OD) determined at 230, 260, 280 and 320 nm against a blank of TE buffer. The concentration of DNA in the extract was then calculated from the absorption at 260nm. The reading at 260nm allows calculation of DNA concentration, since 1 OD unit at 260 nm is equivalent to 50µg/mL for double stranded DNA (Heptinstall and Rapley, 1999 in Naomab, 2004). Therefore, the formula used for calculation was, *DNA conc.* = $A_{260nm} \times 50 \times dilution$

b. Electrophoretic quantification

A 1% agarose gel was used for analysis.

A mixture of 1µl of 6 x gel loading dye and 3µl of each DNA sample was made before loading into the wells of the gel. Five µl of 1kb λ DNA was loaded in two wells and run along the samples. The gel was submerged in a 1 x TBE buffer and run for 1 hour at 100V and then visualized and photographed on a UV imager.

3.3.1.3. PCR amplification and analysis

RADP analysis

PCRs were carried out in a 25µl mixture containing 10xPCR buffer, 2mM MgCl₂, 10mM dNTP mix, 10x Taq DNA polymerase, 10mM primer, double distilled water and extracted DNA. *Table 2* below shows RAPD primers used for the study.

Table 2: RAPD primers used for Bambara groundnut diversity study

	Primer	Sequences 5' to 3'	Annealing temperature(°C)
1	OPA07	GAAACGGGTG	30.8
2	OPAI15	GACACAGCCC	37.2
3	OPB08	GTCCACACGG	37.3
4	OPL12	GGGCGGTACT	38.7
5	OPP04	GTGTCTCAGG	30.6
6	OPP15	GGAAGCCAAC	32.9
7	OPP19	GGAAGGACA	32.7

Microsatellites analysis

PCRs were carried out in a 25µl mixture containing 10xPCR buffer, 2mM MgCl₂, 10mM dNTP mix, 10x Taq DNA polymerase, 10mM primer (reverse and forward), double distilled water and extracted DNA. *Table 3* below shows microsatellite primers used for the study. These primers were provided by Ms Takundwa who developed them specifically for marama bean analysis (Takundwa pers. com, 2009).

Table 3: Microsatellite primers used for genetic diversity study in Bambara groundnut

Primer	Left primer (L)	Right primer (R)
MARA001	GCACAACCAATTTCTGCTT	TCCCTCACTGGCCTATATCC
MARA020	TGTCTTCCCCTCCTCTTCCT	TTGACACTTTGGGACTGCTG
MARA037	GGGAGGATCAATCTTCACCA	TCCGAGAGAAGAGGAGGAAA
MARA038	TGTTGATGAACTAGTGCTAGTGGT	AGCACTCTCCAGCCTCTCA
MARA043	TGTTGATGAACTAGTGCTAGTGGT	AGCACTCTCCAGCCTCTCA
MARA046	GCACTCAGGCAACTGTGCTA	TGGCTGGCACTCTGATTAAG
MARA052	GCACTCAGGCAACTGTGCTA	CACGCTCTCACAAGAAACA
MARA065	TGGTGGTAGGGTGGTGGTAT	CCACTTTTCACAGGCAAACA

The PCR reaction profile used involved an initial denaturation step of 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for 30 sec, an annealing with varying

temperatures depending on specific primers for 60 sec and an extension at 72°C for 2 minutes, a final extension at 72°C for 5 minutes and then held at 40°C. PCR products were analysed by electrophoresis in 2.5% agarose gel (ethidium bromide-stained) in 1X TBE buffer. Samples were run alongside a 100bp DNA ladder used as a standard molecular weight size marker. Gel was visualised under a UV doc system and permanent records obtained. These records were then used for data analysis. Each RAPD/microsatellite band was scored as a binary character 1 for present, zero for absence and 9 for no amplification of DNA sample by looking at the ethidium bromide stained gels observed under a UV doc system.

3.3.2. Nutritional evaluation of the six landraces

Analysis of the harvested seeds was done at the Analytical laboratory (Windhoek – Namibia). Proximate content of the seeds including moisture, protein, fat, ash and carbohydrate was determined by standard methods of Association of Official Analytical Chemists (AOAC).

Moisture determination:

Moisture was determined by weighing 5g of the sample in a covered, flat, aluminium dish and dried to constant weight at 100°C. The formula below was then used to calculate the moisture percentage.

$$\text{Moisture content (\%)} = \frac{\text{Mass of fresh sample} - \text{mass of dry sample} \times 100}{\text{Mass of fresh sample}}$$

Crude protein determination:

Total nitrogen was determined by the Kjeldahl method and the result multiplied by 6.25 to give crude protein. This method involves digestion and distillation techniques, using Macro

Kjeldahl digestion and distillation units. The formula below was used to calculate nitrogen percentage of the sample.

$$\text{Nitrogen content of sample (\%)} = \frac{\text{mL acid} \times \text{Normality of standard acid} \times 0.014 \times 100}{\text{Mass of sample}}$$

Then the formula below was used to determine the crude protein percentage

$$\text{Crude protein content (\%)} = \text{nitrogen content} \times 6.25$$

Ash determination:

Ash was determined by weighing 2g of sample into a dry, tarred porcelain dish and then placed in a muffle furnace at 600°C for 6hours. This was cooled in desiccators and then weighed. The formula below was then used to calculate ash percentage.

$$\text{Ash (\%)} = \frac{\text{Mass of ash}}{\text{Mass of sample}} \times 100$$

Crude fat determination:

Crude fat was determined by weighing out 3g of dried sample in an extraction thimble, the thimble was then placed inside the soxhlet apparatus. The required quantity of solvent was added and connected to the condenser. Heating rate was adjusted and extraction was done for 16hours. The solvent was reclaimed on a boiling water bath and flask was dried at 105°C for 30minutes. The sample was cooled in desiccators and weighed. The formula below was then used to calculate crude fat percentage.

$$\text{Crude fat and oils (\%)} = \frac{\text{Mass of fat}}{\text{Mass of sample}} \times 100$$

Carbohydrate determination:

Total carbohydrate was calculated by difference of other analyzed components.

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ ash} + \% \text{ Nitrogen} + \% \text{ Crude fat and oils})$$

3.4. Survey on acceptability, processing and utilization of Bambara groundnut in Namibia

The survey was done to test the acceptability of products from Bambara groundnut by end-users and assess potential for increased demand and utilization of Bambara groundnut in Namibia. A questionnaire was designed and administered to the farmers and other end-users. The questionnaire (see *appendix D*) was treated confidentially and only for research purposes. It included questions that dealt with:

- The socio-economic background of the interviewee
- Production of Bambara groundnut and other cereal legumes on the farm
- Storage, processing and utilization of Bambara groundnut
- Gender issues and responsibilities related to Bambara production in the household
- Socio-cultural perceptions on Bambara groundnut and how they affect consumption
- Marketing of Bambara groundnut

Targeted for this survey were one region (Omusati Region) and three constituencies within the Region. Five villages within each constituency and five farmers within each village were selected (as advised by the agricultural extension officers). Agricultural extension officer based their selection on villages in which they were aware of bambara groundnut being highly cultivated, consumed and marketed.

3.5. Data analysis

3.5.1. Ecological responses of the six landraces

Calculated parameters, i.e. emergence count, RWC, WUE, individual plant yield and total crop yield, for the different landraces were compared to determine which landrace is better suited for the Namibian environment. Data obtained for the two seasons was compared to test

the effect of heat and cold stresses on the crop's yield. Data for irrigated and non-irrigated were also compared to test for the effect of drought stress on the crop.

Data was analyzed using K independent samples test (Kruskal-Wallis test) in the SPSS statistics 17.0 package.

3.5.2. Genetic diversity of the Bambara groundnut landraces

Each RAPD and microsatellite amplified band was scored as binary character 1 for presence, zero for absence and 9 for no amplification of DNA sample by looking at the ethidium bromide stained gels observed under a UV machine.

Percentage polymorphism was calculated using the formula: $P = \frac{pi}{N} \times 100$, where pi is the number of polymorphic loci and N is the total loci. The genetic diversity within the Bambara population was measured by the Shannon diversity index (H). The index was calculated using the following formula: $H = -\sum p_i \log p_i$. Genetic distance (pair wise matrix between individuals) was constructed using Bray-Curtis similarity, using a Primer5 analysis system. The similarity matrix was subjected to cluster analysis resulting in a polygenic tree.

3.5.3. Nutritional evaluation of the six landraces of Bambara groundnut

Data obtained for protein, carbohydrates, fats and oil were compared to determine if the different landraces had the same nutritive values. Data analysis was done using K independent samples test (Kruskal-Wallis test) in the SPSS statistics 17.0 package.

3.5.4. Survey on acceptability, process and utilization of Bambara groundnut in Namibia

As no alternative products are made from Bambara groundnut (such as milk, cakes, soap, flour, and others) are made or sold in Namibia, data was collected on preference of landraces, cooking methods, seeds and pods storage methods, constraints on crop production and gender contribution to activities done to prepare for planting or selling Bambara groundnut. The qualitative data was presented in forms of graphs.

CHAPTER 4

4. RESULTS

4.1. Ecological responses of the six landraces of Bambara groundnut

4.1.1. Germination

Germination ranged from 50 to 89%, with seeds grown during the cold season having the lowest germination percentage (see *figure 3*).

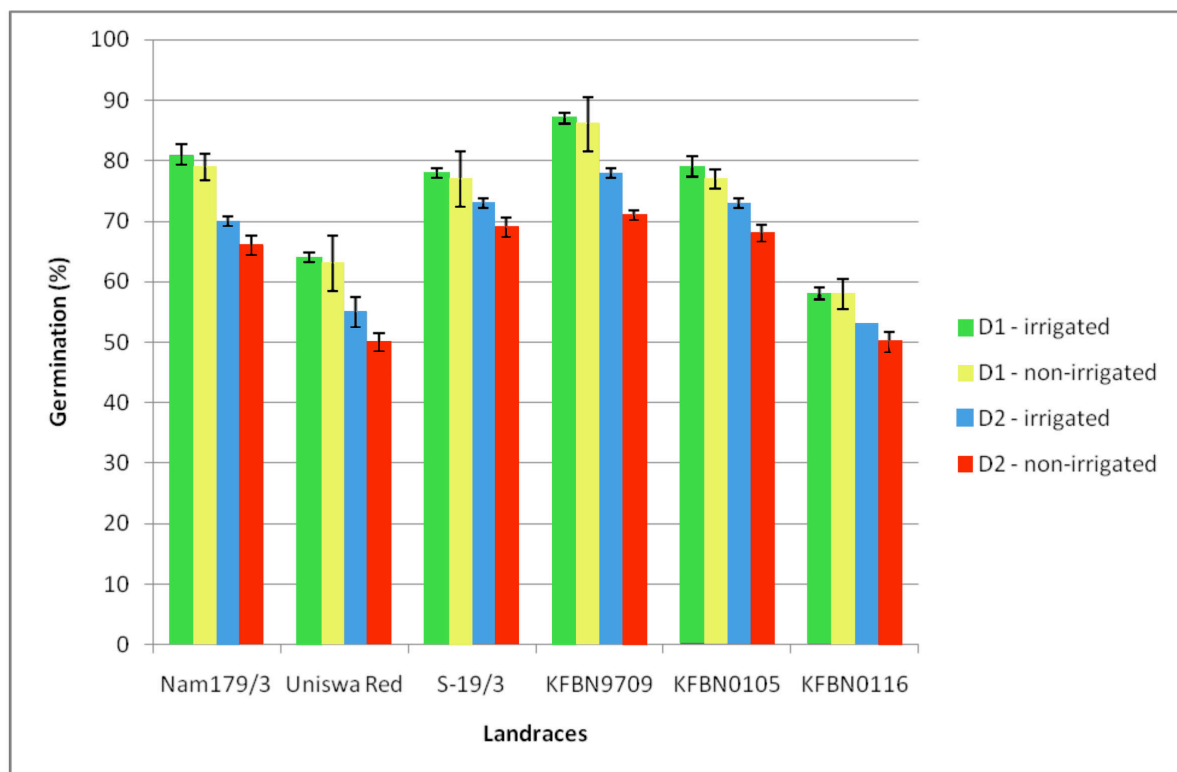


Figure 3: Germination percentage of irrigated and non-irrigated plots of seeds sown during the two seasons.

Figure 3 shows the germination percentage for the two seasons and both irrigated and non-irrigated plots. The figure shows that the irrigated section had a high germination percentage, also that seeds sown in hot season had a high germination percentage than those sown in cold season.

By looking at the figure it is clear that different landraces had different germination percentage. For the first sowing date, the irrigated plot had the highest germination with landrace 4 (KFBN 9709) having the highest germination percentage of 87% followed by landrace 1 (Nam 179/3) of 81% and landrace 6 having the lowest of germination of 58%, a pattern that is clear with non-irrigated plots as well. For the second sowing season, landrace 4 has again the highest germination of 78%, followed by landrace 3 and 5 with 73%, while landrace 6 was again the lowest with germination percentage of 53%.

Using the K independent samples test (Kruskal-Wallis test), the analysis showed that there was a significant difference among the different landraces as far as germination for seeds sown during the hot season was concerned ($P < 0.05$, $\chi^2 = 18.785$, $df = 5$). Using a 2 independent samples test (Mann-Whitney test) showed that the difference was between landrace 1 and landrace 2 ($Z = -2.366$); landrace 1 and landrace 6 ($Z = -2.366$); landrace 2 and landrace 3 ($Z = -2.366$); landrace 2 and landrace 4 ($Z = -2.366$); landrace 2 and landrace 5 ($Z = -2.366$); landrace 3 and landrace 6 ($Z = -2.366$); landrace 4 and landrace 5 ($Z = -2.366$); landrace 4 and landrace 6 ($Z = -2.366$); landrace 5 and landrace 6 ($Z = -2.366$). For the cold season germination, the analysis showed that there was a significant difference among the different landraces as far as emergence count was concerned ($P < 0.05$, $\chi^2 = 20.054$, $df = 5$). Using a 2 independent samples test (Mann-Whitney test) showed that the difference was between landrace 1 and landrace 2 ($Z = -2.366$); landrace 1 and landrace 4 ($Z = -2.366$); landrace 1 and landrace 6 ($Z = -2.366$); landrace 2 and landrace 3 ($Z = -2.366$); landrace 2 and landrace 4 ($Z = -2.366$); landrace 2 and landrace 5 ($Z = -2.366$); landrace 2 and landrace 6 ($Z = -2.366$); landrace 3 and landrace 4 ($Z = -2.366$); landrace 3 and landrace 6 ($Z = -2.494$); landrace 4 and landrace 5 ($Z = -2.366$); landrace 4 and landrace 6 ($Z = -2.494$); landrace 5 and landrace 6 ($Z = -2.494$). analysis showed also that there was a significant difference between the sowing season for all landraces as far as germination is concerned.

4.1.2. Plant water content and water use efficiency

Plant water content was measured as a function of leaf relative water content (RWC).

RWC varied throughout the plant's development stages, but it increased with time. It ranged from 37% to 82%, which showed that the plants did not wilt during the development. The last sowing date, however, showed that the plant was not taking up enough water at the beginning, but the evaporation rate was low which allowed the plants not to wilt.

Table 4: average leaf RWC at different DAS for the first sowing season

	Leaf RWC \pm SE									
	20 DAS		45 DAS		60 DAS		105 DAS		120 DAS	
Nam179/3	56	± 5.310	45	± 2.217	47	± 8.799	50	± 4.049	50	± 4.049
Uniswa Red	48	± 4.785	45	± 2.898	57	± 10.950	58	± 3.428	58	± 3.428
S-19/3	37	± 9.398	45	± 3.894	54	± 9.566	53	± 4.715	53	± 4.715
KFBN9709	55	± 3.330	46	± 3.240	57	± 2.323	53	± 3.122	53	± 3.122
KFBN0105	57	± 9.162	45	± 1.555	46	± 5.543	55	± 3.224	55	± 3.224
KFBN0116	55	± 4.799	47	± 1.683	44	± 10.160	55	± 6.142	55	± 6.142

Table 4 shows the average leaf RWC for the different landraces sown during the last sowing date at different DAS for the first sowing date. The RWC increased with time and became constant towards 105 DAS. At maturity of the plant, landrace 6 had the highest value of 82% followed by landrace 1 and landrace 2 with RWC value of 74%. No significant difference was found among the different landraces ($P > 0.05$, $\chi^2 = 2.588$, $df = 5$) for irrigated plants and no significant difference was found between irrigated and non-irrigated plants for all landraces.

Table 5: Average leaf RWC at different DAS for the second sowing season

	Leaf RWC \pm SE									
	20 DAS		45 DAS		60 DAS		105 DAS		120 DAS	
Nam179/3	0	± 0.000	0	± 0.000	49	± 1.548	49	± 1.548	74	± 5.448
Uniswa Red	0	± 0.000	0	± 0.000	47	± 2.179	47	± 2.179	77	± 0.000
S-19/3	0	± 0.000	0	± 0.000	47	± 1.887	47	± 1.887	83	± 2.933
KFBN9709	0	± 0.000	0	± 0.000	58	± 4.191	58	± 5.132	71	± 5.622
KFBN0105	0	± 0.000	0	± 0.000	54	± 2.869	54	± 2.869	73	± 2.721
KFBN0116	0	± 0.000	0	± 0.000	49	± 2.056	49	± 2.056	79	± 5.344

Table 5 shows the average leaf RWC for the different landraces sown during the last sowing date at different DAS for the last sowing date. Until after 45 DAS, the plants were still germinating, so no analysis could be done on them. At maturity landrace 3 had the highest RWC of 83% followed by landrace 6 with RWC of 79%. No significant difference was found among the different landraces ($P > 0.05$, $\chi^2 = 4.639$, $df = 5$) for irrigated plants and no significant difference was found between irrigated and non-irrigated plants for all landraces. Water use efficiency was measured as a function of soil moisture content.

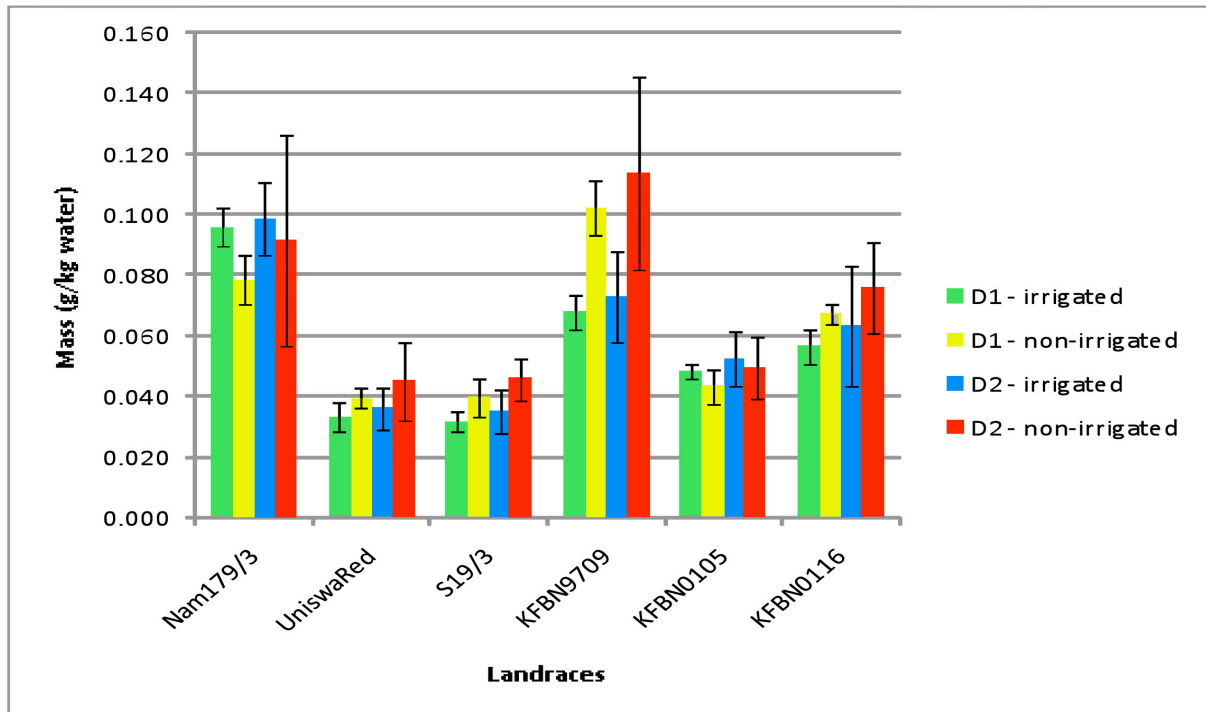
**Figure 4:** WUE of six landraces of Bambara groundnuts for two sowing seasons in both irrigated and non-irrigated plots.

Figure 4 shows the calculated WUE efficiency of the different landraces. As it can be seen, the seeds grown in the cold season produced high biomass per kg water compared to the ones grown in hot season. Statistically, however, there was no significant difference. Although there was no significant difference between irrigated and non-irrigated plots, data strangely shows that for some of the landraces, more biomass was produced from non-irrigated plots than irrigated plots per kg of water used.

4.1.3. Harvest

Seeds produced by individual plants ranges from 0.218g to 45.825g for irrigated plants and 0.221g to 46.769g for non-irrigated plants. The plants sown during the winter season produced little compared to the ones sown in the hot season.

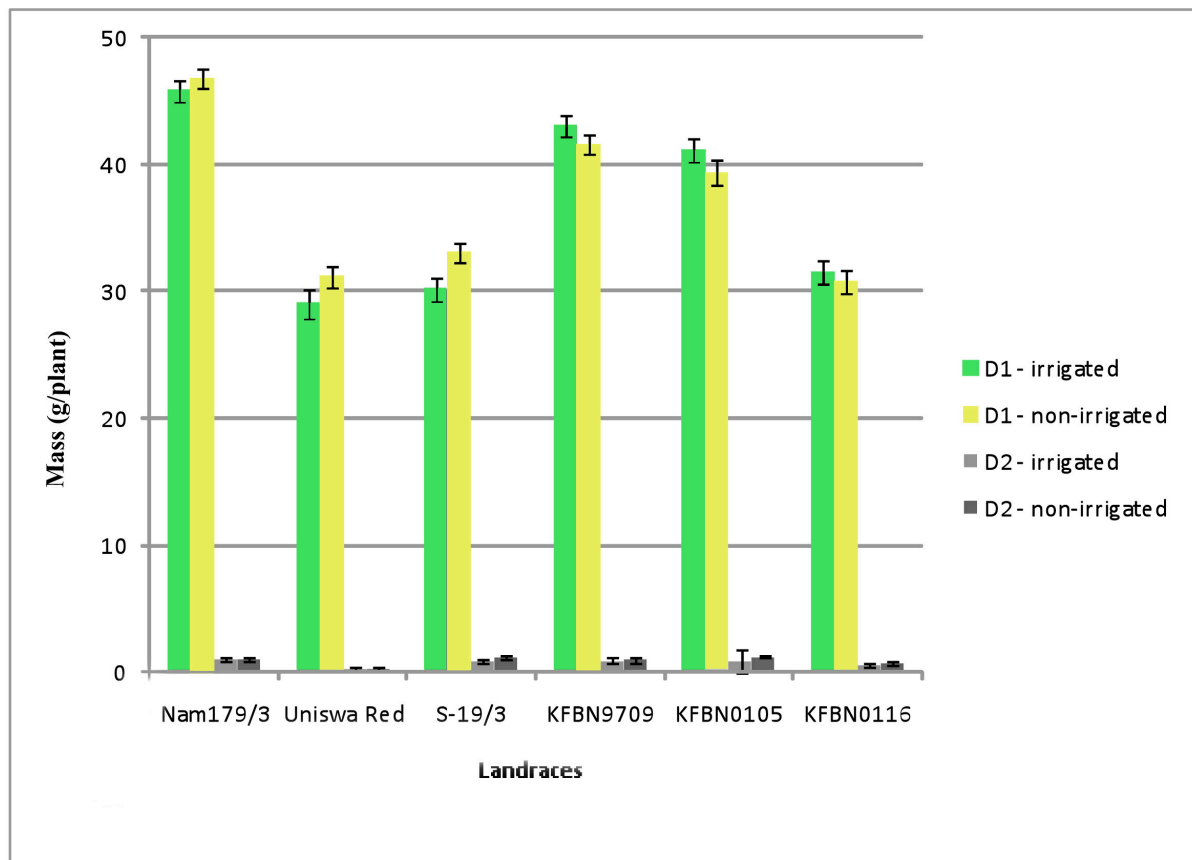


Figure 5: Seed yield from individual plants for both sowing season for irrigated and non-irrigated plots.

Figure 5 shows the average mass of seeds harvested from individual irrigated plants. For the hot season yield, landrace 1 had the highest individual production of 45.825g/plant and landrace 2 had the lowest of 29.025g/plant. Also for the cold season, landrace 1 had the highest of 0.981g/plant and landrace 2 the lowest of 0.218g/plant.

Analysis showed that for the individual plant yield there was no significant difference among the six landraces ($\chi^2 = 4.960$, $df = 5$, $P > 0.05$) as well as between irrigated and non-irrigated plots. However, analysis also showed that there was significant difference for the landraces based on the different sowing dates.

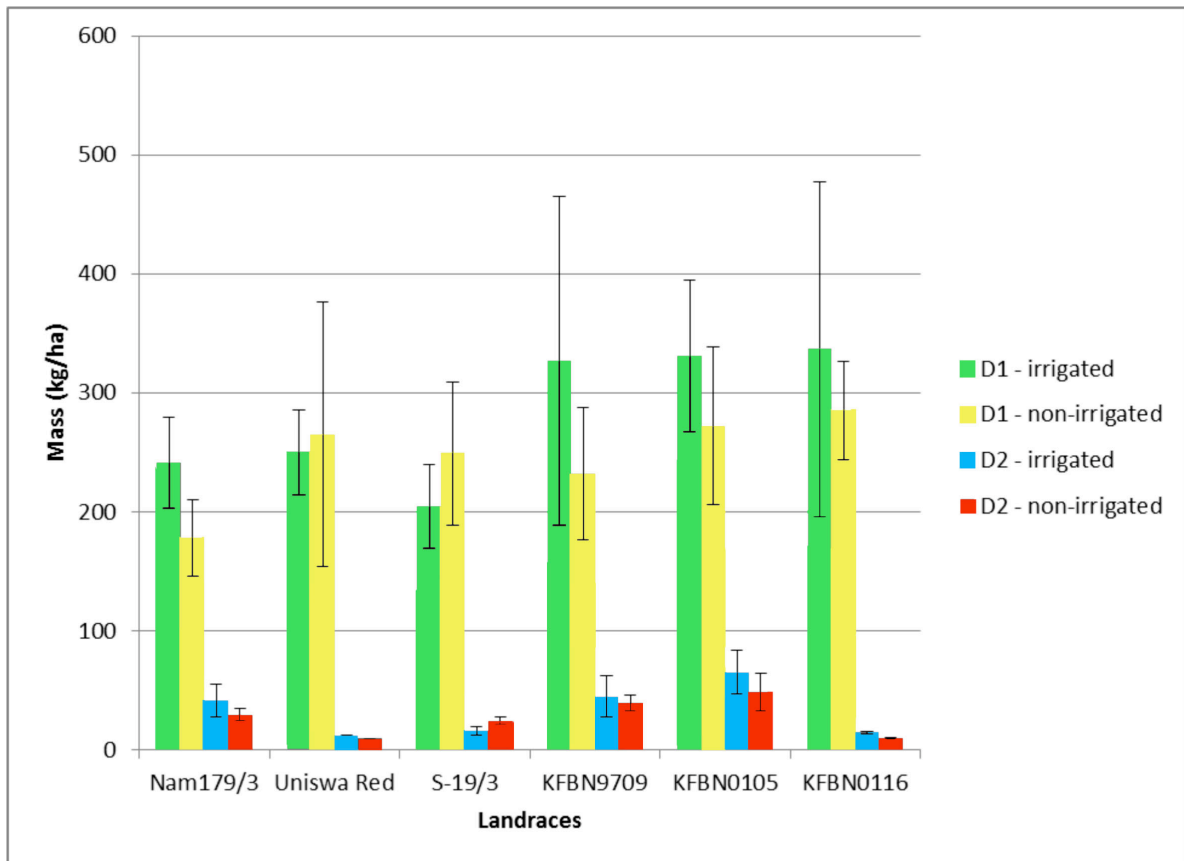


Figure 6: Average crop yield for both hot and cold season for six landraces irrigated and non-irrigated plots.

Figure 6 shows the average crop yield per plot for each landrace. The yield ranged from 336.535kg/ha to 11.778kg/ha. No significant difference was shown between irrigated and non-irrigated plots. However, when the harvest for the different landraces were compared based on the different sowing seasons, all landraces showed a significant difference. landrace 1 ($\chi^2 = 9.269$, $df=2$, $P<0.05$); landrace 2 ($\chi^2 = 9.846$, $df=2$, $P<0.05$); landrace 3 ($\chi^2 = 8.000$, $df=2$, $P<0.05$); landrace 4 ($\chi^2 = 9.269$, $df=2$, $P<0.05$); landrace 5 ($\chi^2 = 9.269$, $df=2$, $P<0.05$) and landrace 6 ($\chi^2 = 9.302$, $df=2$, $P<0.05$).

4.2. Genetic diversity of the six Bambara groundnut landraces

4.2.1. RAPD analysis

All primers used were able to amplify bands; and produce polymorphic bands.

Table 6 below shows the calculated polymorphism and diversity index using RAPD primers. The calculated percentage polymorphism was high for all primers, ranging from 57% to 100%. A total of 119 bands were amplified and out of those 101 (85%) were polymorphic bands while the remaining 15% were monomorphic bands. The calculated diversity index was 0.426, showing low diversity among the landraces.

Table 6: Percentage polymorphism and genetic diversity measure using RAPD primers

	Primer	N° of amplified bands	N° of polymorphic bands	% polymorphism	H
1	OPA07	17	17	100	0.418
2	OPAI15	19	13	68	0.301
3	OPB08	16	16	100	0.408
4	OPL12	24	18	75	0.530
5	OPP04	14	8	57	0.355
6	OPP15	10	10	100	0.313
7	OPP19	19	19	100	0.659
Total		119	101	85	
				H'	0.426

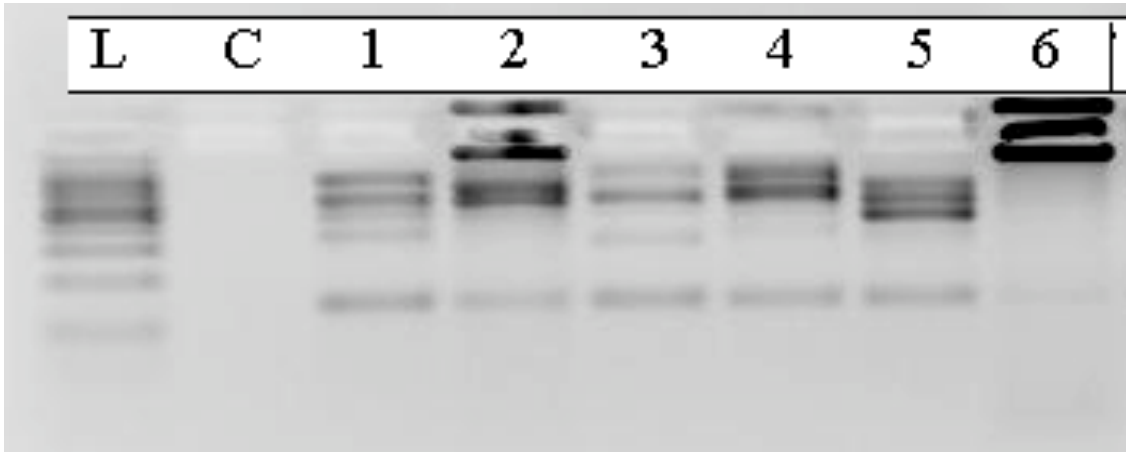


Figure 7: Primer OPB08 gel electrophoresis. Sample 1-6 are the 6 landraces of Bambara groundnut, lane L contains a 100 bp ladder. The gel was 2.5 % agarose. Electrophoresis was at 90V for 1 hour 30 minutes.

Figure 7 shows a 2.5% gel that was ran for 1hour 30mintes at 90V for OPB08 primer. The gel also shows that there are different scorable bands for the different landraces.

Table 7 below shows the similarity matrix for the six landraces of Bambara groundnut. Some of the landraces are closely similar with the highest similarity at 95.7% between landrace 1 and landrace 3, second highest at 84.4% between landrace 2 and landrace 3, and lowest at 36.4% between landrace 5 and landrace 6. These similarities are also reflected on the dendrogram (Figure 4), where they are grouped.

Table 7: Bray-Curtis similarity matrix using PRIMER 5 software for the six landraces of Bambara groundnut using RAPD markers

	Nam179/3	Uniswa Red	S-19/3	KFBN9709	KFBN0105	KFBN0116
Nam179/3						
Uniswa Red	84.4					
S-19/3	95.7	84.4				
KFBN9709	82.9	65.0	82.9			
KFBN0105	78.0	75.0	73.2	55.6		
KFBN0116	52.6	54.1	52.6	48.5	36.4	

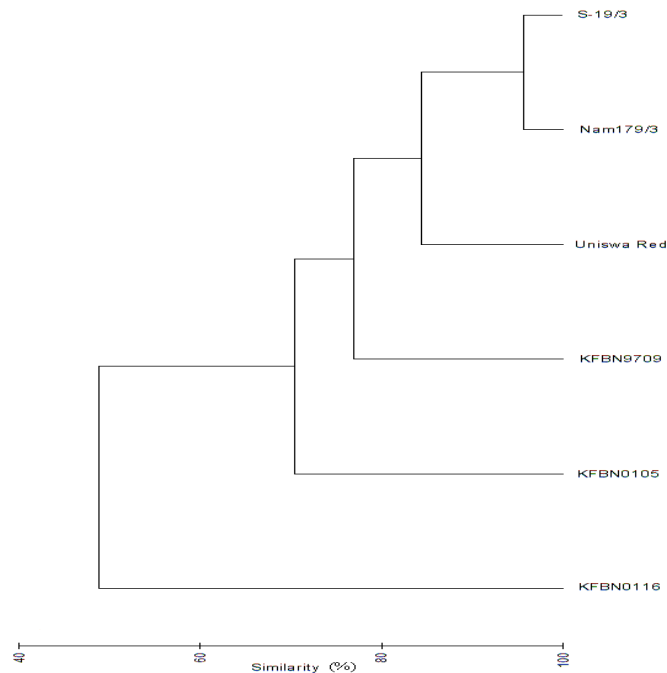


Figure 8: Dendrogram of the six landraces of Bambara groundnut using RAPD markers

Figure 8 shows the cluster analysis of the six landraces of Bambara groundnut using RAPD markers. As it can be seen, the landraces are closely related/similar, indicating low diversity.

4.2.2. Microsatellite analysis

All primers used were able to amplify bands; however, out of the eight primers used four produced polymorphic bands.

Table 8 below shows the calculated polymorphism and diversity index using microsatellite primers. The calculated percentage polymorphism was low ranging from 14% to 100%. A total of 59 bands were amplified, of which 17 (29%) were polymorphic bands, while the rest 71% were monomorphic bands.

Table 8: Percentage polymorphism and genetic diversity measure using microsatellite primers

	Primers	N°. of amplified bands	N°. of polymorphic bands	%polymorphism	H
1	MARA001	6	0	0	0.000
2	MARA020	6	0	0	0.000
3	MARA037	9	9	100	0.183
4	MARA038	7	1	14	0.130
5	MARA043	7	1	14	0.130
6	MARA046	6	0	0	0.000
7	MARA052	6	0	0	0.000
8	MARA065	12	6	50	0.301
Total		59	17	29	
				H'	0.093

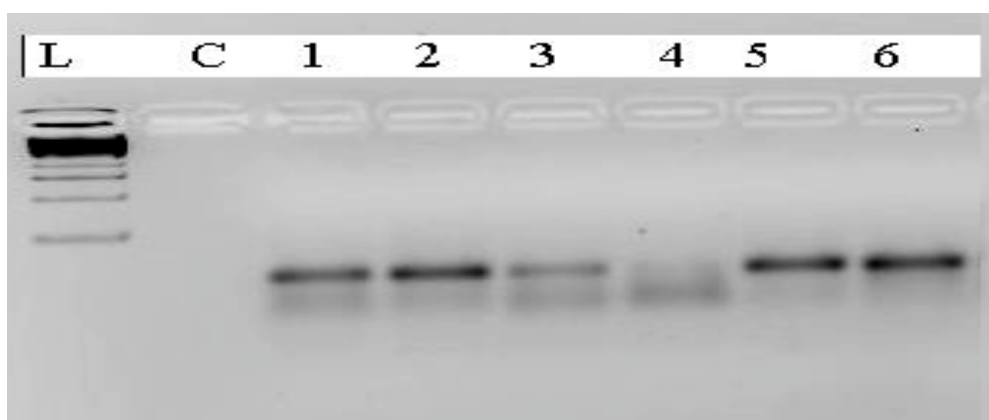


Figure 9: Primer MARA 037 gel electrophoresis. Sample 1-6 are the 6 landraces of Bambara groundnut, lane L contains a 100 bp ladder. The gel was 2.5 % agarose. Electrophoresis was at 90V for 1 hour 30 minutes.

Figure 9 shows a 2.5% gel that was ran for 1 hour 30 mintes at 90V for MARA037 primer. The gel shows that there are different scorable bands for the different landraces.

Table 9 below shows the similarity matrix for the six landraces of Bambara groundnut. The landraces are closely similar. Similarity ranges from 73.7% (between landrace 4 and landrace 5) to 95.7% (between landrace 1 and landrace 2). These similarities are also reflected on the dendogram (Figure8), where they are grouped.

Table 9: Bray-Curtis similarity matrix using PRIMER 5 software for the six landraces of Bambara groundnut using microsatellite markers

	Nam179/3	UniswaRed	S19/3	KFBN9709	KFBN0105	KFBN0116
Nam179/3						
UniswaRed	95.7					
S19/3	90.0	85.7				
KFBN9709	84.2	80.0	94.1			
KFBN0105	90.9	87.0	80.0	73.7		
KFBN0116	84.2	80.0	94.1	87.5	84.2	

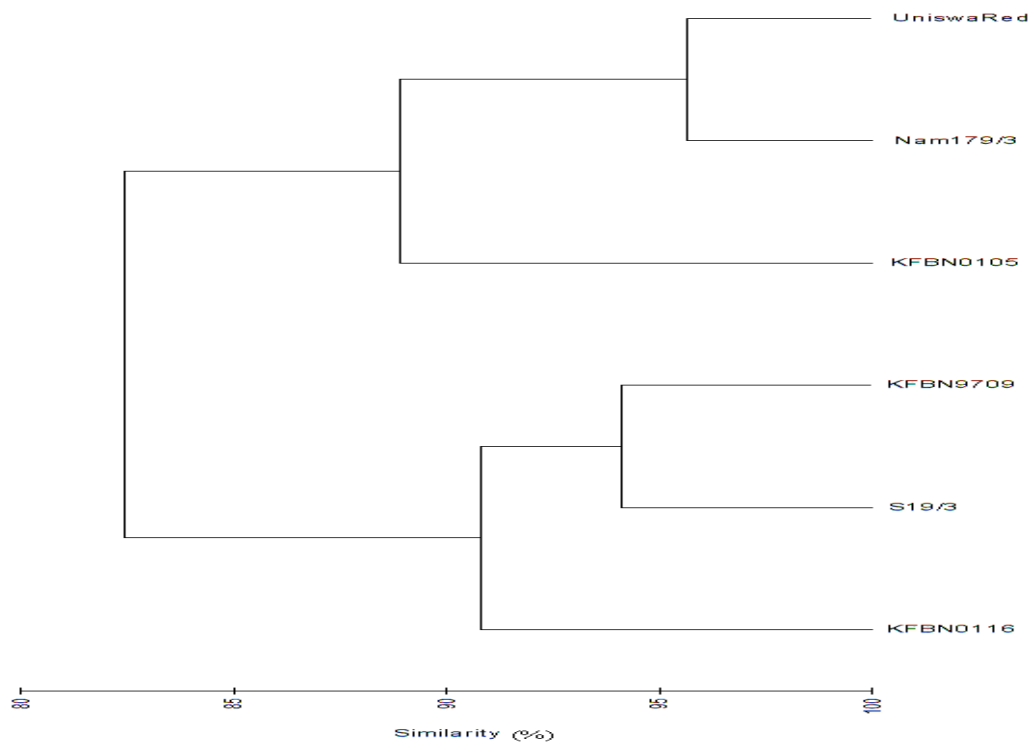


Figure 10: Dendrogram of the six landraces of Bambara groundnut using microsatellite markers

Figure 10 shows the cluster analysis of the six landraces of Bambara groundnut using microsatellite primers.

4.3. Nutritional evaluation of the six landraces of Bambara groundnut

Proximate analysis was done on seeds harvested from both irrigated and non-irrigated plots. Carbohydrate contents were shown to be the most in all seeds (*Figures 10 and 11*). Data analysis showed no significant difference among the landraces for the different nutrient contents.

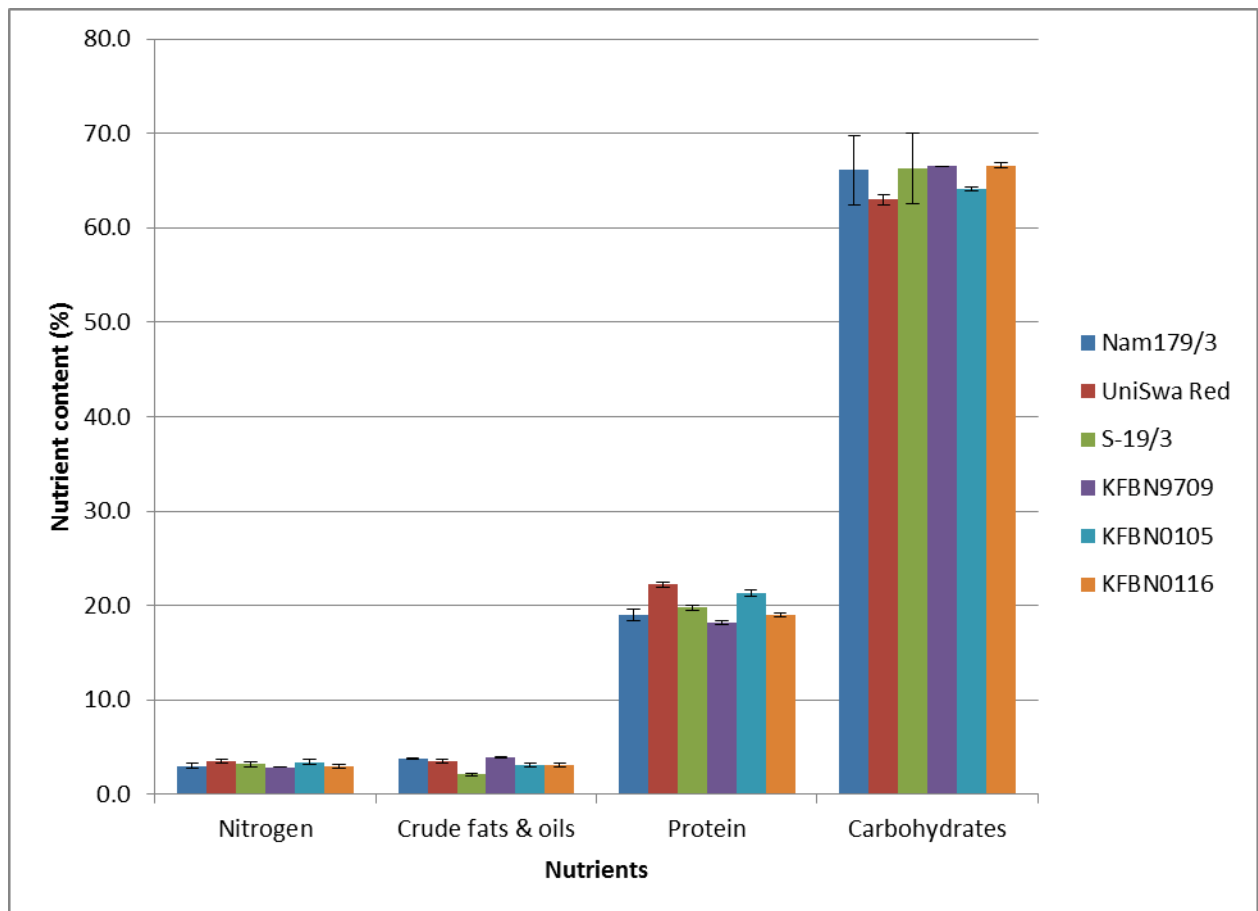


Figure 11: Nutrient content for seeds harvested from seeds sown in summer in the irrigated plots

Figure 11 shows the nutrient content of the six landraces from irrigated plots. The analysis showed that the seeds have high carbohydrate content ranging from 63.0-66.6% and low nitrogen content ranging from 2.9-3.5%.

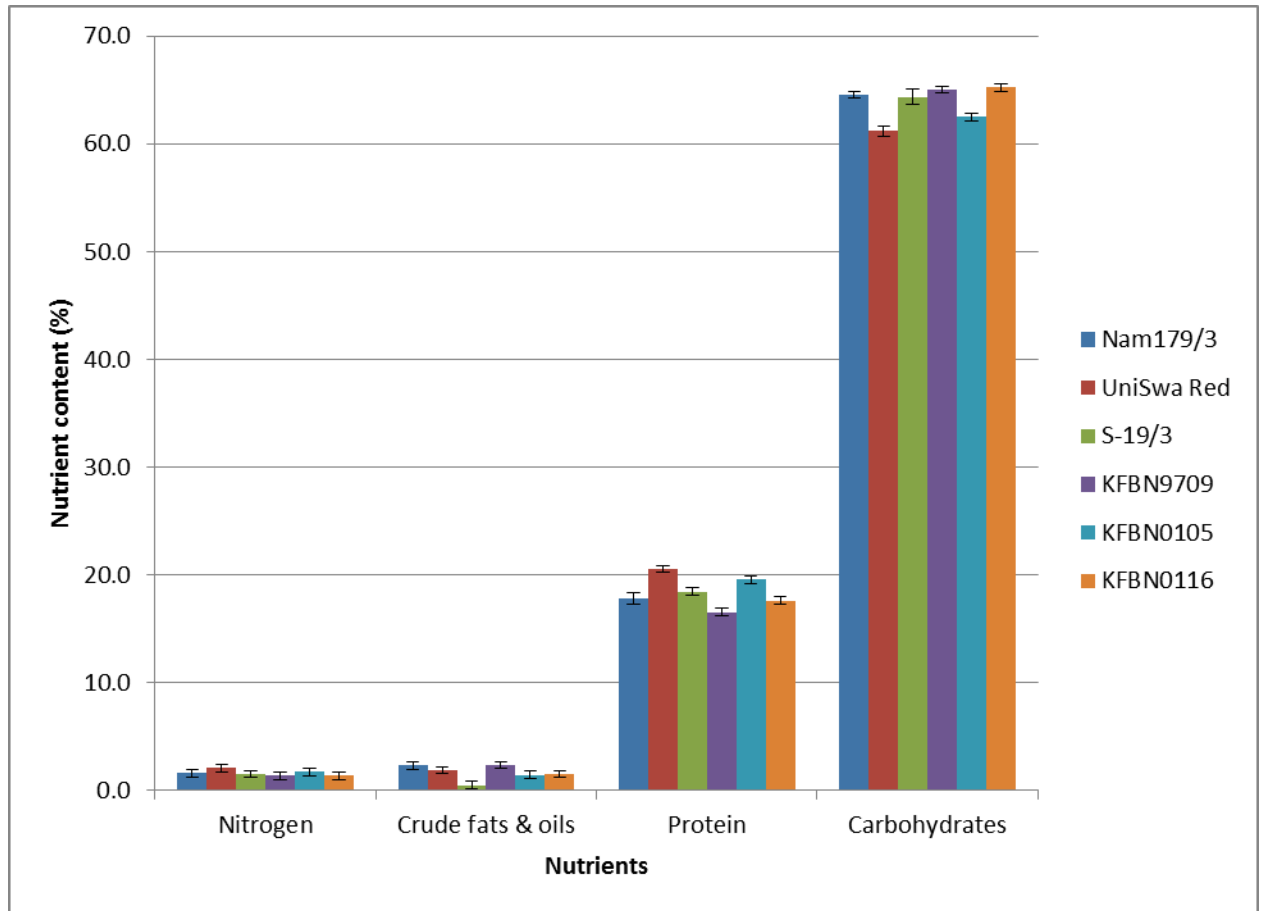


Figure 12: Nutrient content for seeds harvested from seeds sown in summer in the non-irrigated plants

Figure 12 shows the nutrient content of the six landraces from irrigated plants. Also for these seeds, analysis showed that the seeds have high carbohydrate content ranging from 61.2-65.2% and low nitrogen content ranging from 1.4-2.1%.

4.4. Survey on acceptability, processing and utilization of Bambara groundnut in Namibia

The survey showed that in Namibia, Bambara groundnut is not processed into other products such as flour or milk. The majority of consumers interviewed preferred to eat the seeds boiled while still fresh (67%) and few (33%) stated that they preferred it boiled but once dried cooking is longer and requires more fire wood compared to that needed for freshly boiled pods.

The survey also outlined the numerous activities involved from growing Bambara groundnut to marketing it and also the involvement of individuals based on genders in these different activities (*Figure 13*).

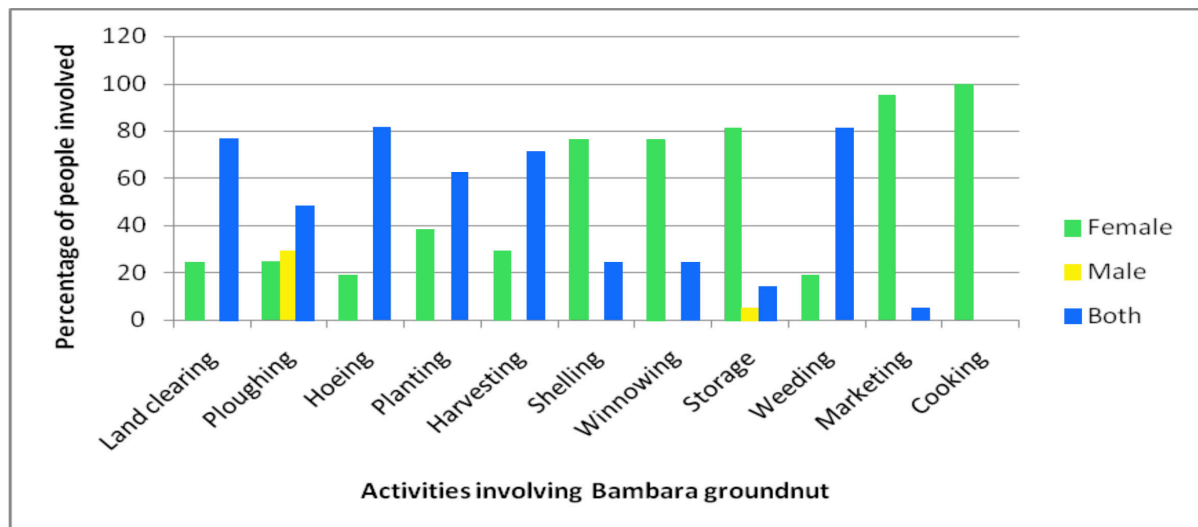


Figure 13: Gender involvement in the different activities to prepare bambara groundnuts either for sowing or marketing or storing.

Figure 13 shows the different activities and the gender contribution for these activities. Gender involvement is widely spread, however, women are mostly the ones involved especially when it comes to storage of seeds or pods (81%), marketing (95%) and cooking (100%).

The survey showed also that the production of Bambara groundnut has some constraints.

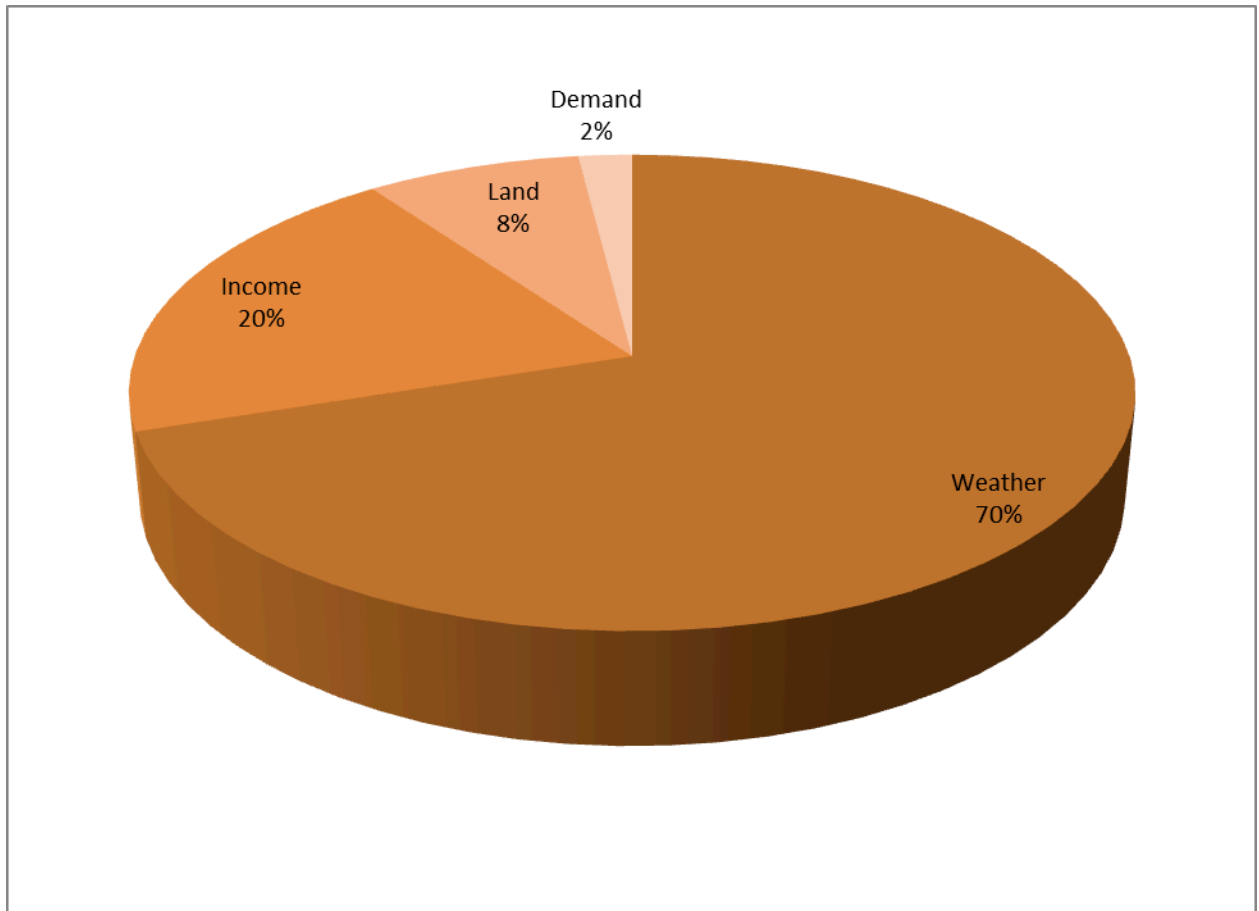


Figure 14: Constraint factors on Bambara groundnut production in Namibia

Figure 14 shows the constraint factors on Bambara groundnut production. The major constraint that the farmers seem to be facing lately is poor weather (70%), while demand of the crop is considered as a minor constraint (2%) to the production.

The survey also showed that in Namibia storage of seeds/pods is still done in the traditional way

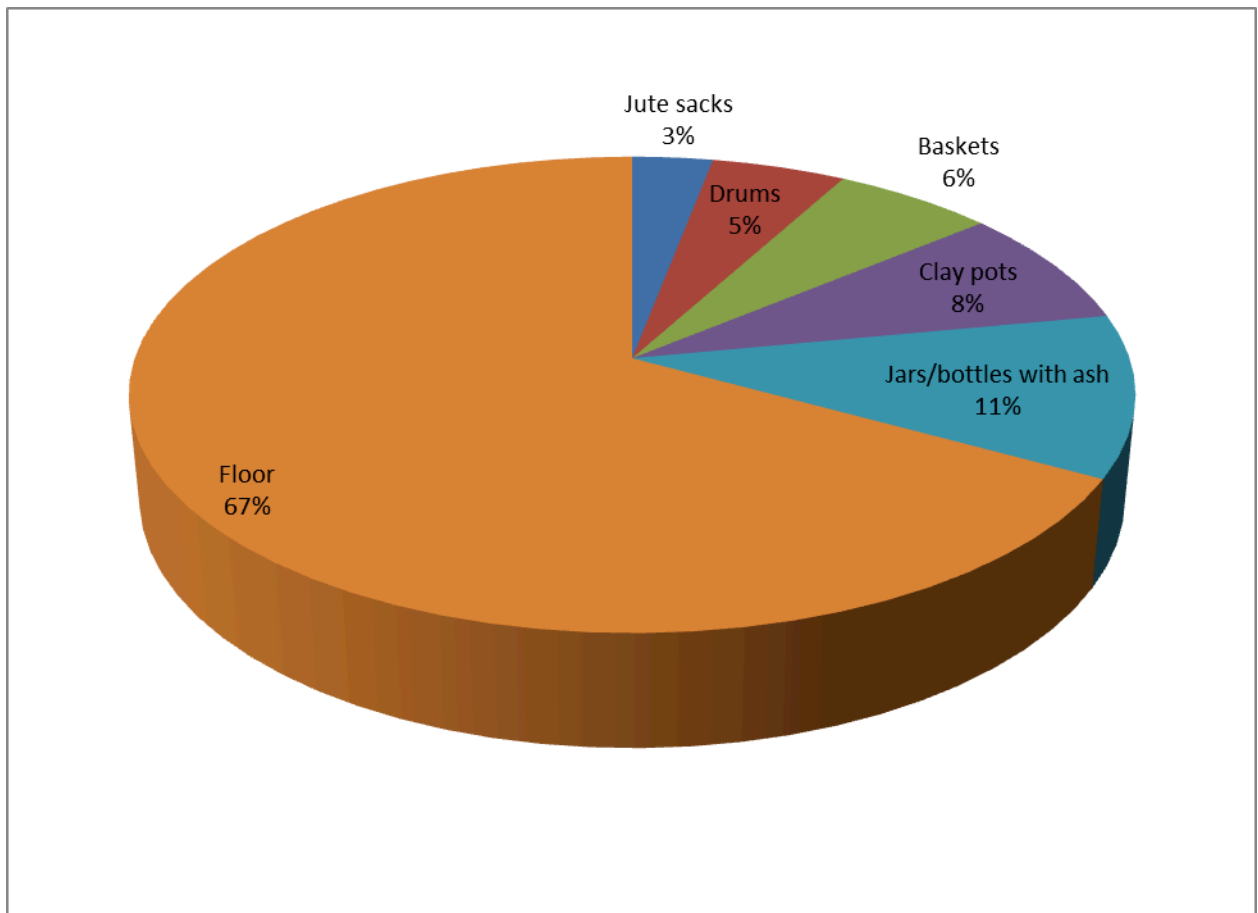


Figure 15: Type of storage of Bambara groundnuts used in Namibia

Figure 15 shows the type of storage used by farmers in Namibia. The most popular storage technique is placing seeds/pods on the floor (67%), while the least is placing them in jute sacks (3%).

The survey also obtained information on seeds that are mostly found with farmers. These are the seeds that farmers prefer to others due to their end results (harvest).

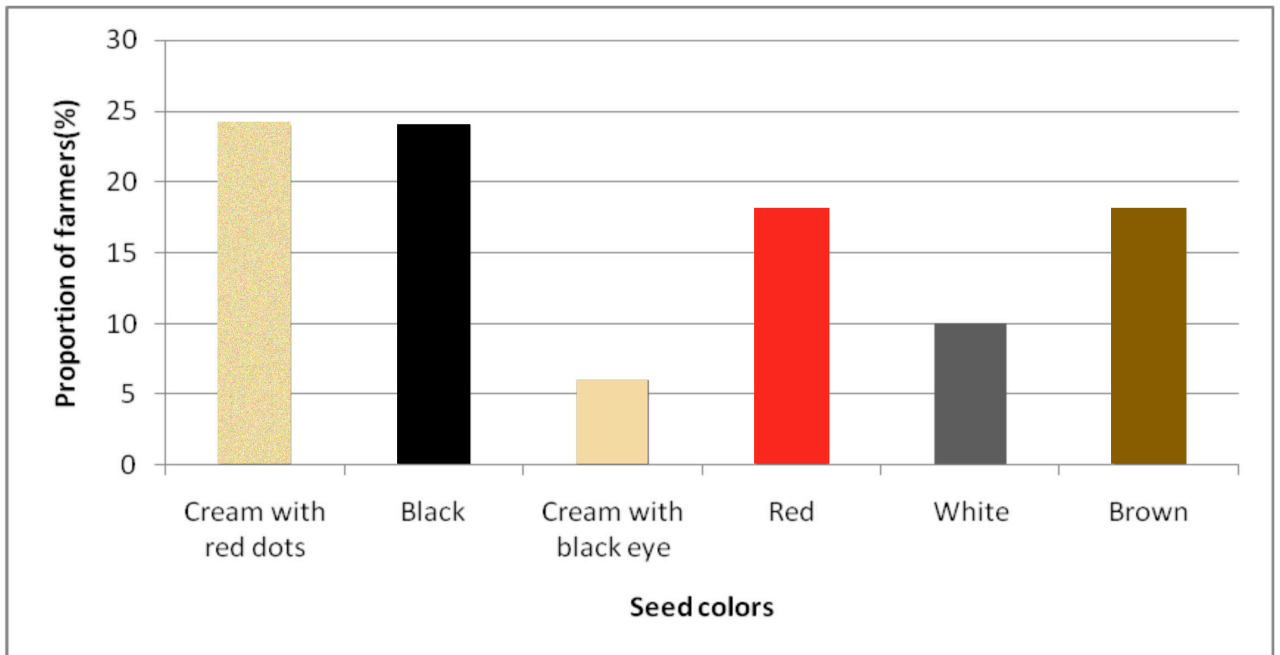


Figure 16: Seed color preferred by farmers in Namibia

Figure 16 shows the seeds that are mostly preferred by farmers. Black seeds and cream with red dots are the most preferred while, cream with black eye seeds are least preferred

CHAPTER 5

5. DISCUSSION

5.1. Ecological responses of the six landraces

The different landraces in this study were subjected to two different sowing dates and irrigation treatments.

The different sowing dates were considered to examine the effect of changing climatic parameters on the growth and yield of Bambara groundnut. The seeds sown earlier in the season showed better growth patterns than the ones sown later. As was observed by Makanda, Tongoona, Madamba, Icishahayo and Derera (2009), there was a great significant difference among the sowing dates for germination, flowering, and yield. The seed sown in the hot season germinated before and gave better germination percentage than the ones sown in the cold season (*Figure 3*). This observation is consistent with the review done by Linneman and Azam-Ali, 1993, who reported that low temperatures delay germination and seedling emergence, with some seeds remaining dormant, therefore, decreasing the plant count per plot at harvest. The low germination of seeds sown in the cold season, decreased the yield of the crop (*Figures 5 and 6*), while the ones sown in the hot season produced more. This observation is also consistent with the findings of Collinson, Sibunga, Tarimo and Azam-Ali, (1996).

The study for moisture tolerance of the crop was done by introducing an irrigation system in which the field was divided into irrigated and non-irrigated sections (plots). Although little significant difference was found between the irrigated and non-irrigated plots, most landraces produced more seeds in irrigated plots than non-irrigated ones for seeds sown in hot season, while all landraces produced more in irrigated plots than non-irrigated plots. Landrace KFBN

0116 produced the most in both irrigated and non-irrigated plots (337kg ha^{-1} and 284kg ha^{-1} , respectively) for the hot season followed by KFBN 0105, which also produced the most during the cold season.

RWC is an appropriate measure of plant water status in terms of physiological consequence of cellular water deficit. It can be described as a measure of water deficit in plants (Barr and Weatherly, 1962). It estimates current water content of the sampled leaf tissue relative to the maximum water content it can hold at full turgidity. Normal values range between 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves (Barr and Weatherly, 1962). The results obtained for RWC for the landraces range from 46% to 60%, which does not show very turgid plants, but it also indicated that the plants were not dehydrated or drying. Although during the rainy season one would expect the values to be higher than the values for winter or dry season, the values obtained for the above mentioned different seasons show no significant difference.

Water use efficiency of the crop clearly shows that the seeds grown in the cold season produced high biomass per kg water used compared to the ones grown in hot season. Although there was no significant difference between irrigated and non irrigated plots, data strangely shows that for some of the landraces, more biomass was produced from non-irrigated plots than irrigated plots per kg water used. This emphasises the fact that the crop is adapted to harsh climatic conditions and have a mechanism for controlling the water conservation regardless of the season it grows in.

Landraces 4, 5 and 6 (KFBN 9079, KFBN 0105 and KFBN 0116 respectively) did well in both hot season and cold season, they can be considered as the ones best suited for the Namibian climate. Based on the outputs from BAM nut Model – Crop Biomass and pods Models in Azam-Ali et.al, 2001, these three landraces can be classified from suitable to moderately suitable based on their yields.

5.2. Genetic diversity of the six landraces of Bambara groundnuts

This study used RAPDs and microsatellites to study the genetic diversity of six landraces of Bambara groundnut. DNA polymorphism was detected, although not all primers were able to produce polymorphic bands (*table 6* and *table 8*). The polymorphism can be due to nucleotides positions or variations present in DNA (Takundwa, 2010, pers. Com.). RAPDs used gave high polymorphism than microsatellites; this may be that RAPDs are a better choice of markers for Bambara groundnuts genetic study, or that the used microsatellites are specific for marama but not Bambara groundnuts. Similarity data calculated for the six landraces of Bambara groundnuts using both RAPDs and microsatellites were generally high, ranging from 36.4% to 100%. These similarities, calculated by the Bray-Curtis index, are reflected by the overall intra-population diversity as shown by the dendrogram (*Figures 8* and *10*). Low Shannon diversity index values of 0.426 (RAPDs) and 0.093 (microsatellites) indicate a low diversity among the landraces, as stated by King and Schaal that indices values close to zero indicate low genetic diversity. Therefore, these calculated values also support the high similarity within the Bambara groundnuts population. The close relationships of the landraces can be due to the fact that they originated from the same area, since it is believed that the centre of origin for Bambara groundnut is Bambara, near Timbuktu in Central Mali, West Africa (de Kock). This implies that the different landraces may have originated from one or similar founding populations. The similarities may also be due to the similarity of phenotypic traits, which is the case for landrace 1 and landrace 4, which may be the same landraces with different names, due to the informal naming system as explained by Massawe, Dickinson, Roberts and Azam-Ali, 2002, who state that landraces acquire names based on the testa colour, place where they are grown or markets from where the seeds are purchased, which may be unrelated to origin or areas of cultivation. An informal method of naming such

as this may lead to one landrace having more than one single name. The similarities can also be brought on by interbreeding of the crop.

5.3. Nutritional evaluation of the six landraces

The tested Bambara groundnut seeds are high in nutrient such as protein (18.2 – 22.2%) and carbohydrates (63 – 66.6%) with traces of extractable fats and oils (2.1-3.9%), which is in accordance to the work previously done by Brough and Azam-Ali (1992), who reported that Bambara groundnut seed makes a balance food as it contains sufficient quantities of carbohydrates (63%), protein (16.25%) and fats (6.3%). The tested Bambara groundnut did not show any difference in their nutrient composition, which means that their morphological differences do not have anything to do with nutrient composition.

Although Bambara is not grown on a large scale in Namibia it is a big part of diet in the northern part of the country, the crop however, is limited to other parts of the country.

5.4. Survey on acceptability, processing and utilization of Bambara groundnut in Namibia

The results obtained by the survey were clearly an indication of how little Bambara groundnut is utilized and processed in Namibia.

Storage of seeds or pods after harvesting is not a sophisticated process in Namibia. After harvesting, the pods that are not eaten fresh are sun-dried. These pods are then stored in the seed room in bags, baskets, drums or clay pots until seeds are needed. Some farmers store shelled seeds in airtight jars, after mixing the seeds with ash to inhibit small insect from

infesting them. If grain production is not too high, some farmers mix the Bambara seeds with the grain and then store them in a grainer. However, most farmers just place the pods on bare floors after harvesting until they are needed.

Namibia seems to have quite a lot of landraces of Bambara groundnut; however, farmers seemed to be more interested with black seeds, reason given to be that “they produce more”. They are aware that they can get other types from research institutions; however, they prefer what they are used to and know well. The farms were all family owned and the fields were small, which contribute, although lightly, on constrains to increase of Bambara groundnut production. Farmers are more comfortable with crops that bring them high income and the ones that they have been dealing with for as long as their grand-fathers and fathers owned the farms, crops such as Mahangu (pearl millet), maize, field beans, and others. Bambara is allocated a very small portion of the field or intercropped with other crops, which is consistent to some parts of Africa, where the crop is grown under traditional low input agricultural systems (Massawe, Mwale, Azam-Ali & Roberts, 2005).

The farmers also find producing Bambara on a larger scale to be “risky” since only people in the Northern part of the country are the only ones familiar with the crop. This also contributes to constrain to increase production.

The acceptability of products from Bambara was not studied, since all farmers and consumers interviewed do not process Bambara groundnut into alternative products. In Namibia Bambara is eaten fresh or dried no alternative products such as milk, pup or other has ever been produced, while in East Africa milk was produced from seeds and dry seeds are ground into powder which is used to make bread (Alakali and Satimehin, 2007).

CHAPTER 6

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Bambara groundnut has potential to contribute to food security as it not only proves to be balanced diet crop, but also a crop that is tolerant to conditions mostly found in areas threatened by low food production.

Although, study showed that there was no significant difference between irrigated and non-irrigated plots, data collected during the previous years with high rainfall showed that the crop doesn't grow well or produce enough with high soil moisture content. Combined with the data showing that the crop produces high yield during the hot season, it is important that sowing of seeds be done at the beginning of the season to allow maximum germination of seeds.

The study with RAPDs and microsatellites showed that there is low genetic diversity among the six different landraces of Bambara groundnut and the diversity found was sufficient to be reflected phenotypically. The low diversity is not reflected on production level as well as growth and development of the crop

This study also showed that these two techniques are efficient and economical to do genetic diversity study for Bambara groundnut, and that microsatellites developed in one species can be used in another, especially if the species in question are closely related.

Nutrient quantities of the seeds from the different landraces ranged within the normal amount as specified by literature review and there was no significant difference among the six landraces which agrees with the hypothesis.

Although Bambara groundnut is becoming a socio-economic crop in most of the Southern Africa countries, in Namibia it is still not well known. Consumers do not mind what type of landrace they are eating as long as it is Bambara groundnut; however, they are not aware of the different products that can be made from the seeds.

6.2. Recommendations

Bambara groundnuts have a good water use efficiency where by production is high with low water usage. A study should be carried out to understand the water preservation mechanism of the crop and what triggers it. The study should look at the gene that is responsible for the WUE mechanism and try to incorporate it in the landraces that are capable of producing high yields to allow them to grow in harsher environments.

Namibia has a large variety of Bambara groundnut; it is important to do a genetic diversity study on all of them especially the ones considered to be wild and not only concentrate on the varieties that are mostly known to the local farmers. There is a need for producing specific markers for Bambara groundnut; this will make the study more accurate. A quantitative trait

loci study should also be done since this is important to allow the identification of the loci associated with the good traits for genetic enhancement of the crop.

For further nutritional evaluation studies, it will be also good to look at the different amino acids, vitamins and other nutrients within the seeds, and also look at the effect of cooking on the levels of these nutrients.

Bambara is a staple food that should be introduced to the population in general. A study should be carried out on the best way to integrate this among the already known food such as Mahangu and maize. Furthermore, it will be encouraged to instruct the farmers as well as consumers on the different ways of processing Bambara, both for home consumption or for those who are willing to market the products.

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APPENDICES

APPENDIX A: Composition of buffers and solutions

10XTBE buffer

For 1L

108g Tris

40ml 0.5M EDTA (pH 8.0)

pH adjusted to 8.3 with NaOH

Volume adjusted to 1L with ddH₂O

TE buffer

10mM Tris-HCl (pH 8.0)

1mM EDTA

0.5M EDTA (pH 8.0)

For 1L

186.1g EDTA (Na salt) dissolved in 800ddH₂O

20.0g NaOH added while stirring

pH adjusted to 8.0 with NaOH

Volume adjusted to 1L

APPENDIX B: Questionnaire

Questionnaire code: /.../.../.../.../

Interview code: /.../.../.../.../

BAMLINK

Molecular, Environmental and Nutritional Evaluation of Bambara Groundnut (*Vigna subterranea* L. Verdc.) for Food Production in Semi-Arid and India

Questionnaire on production, processing, marketing and utilisation of Bambara groundnut in Namibia

This questionnaire is solely for research purposes. Information provided will be treated as confidential. Thank you.

A. SOCIO-ECONOMIC BACKGROUND AND GENERAL INFORMATION

- (i) Respondent code number
- (ii) Name of enumerator
- (iii) Date of interview
- (iv) Name of respondent
- (v) Contact address (H No.)
- (vi) Name of town/village
- (vii) District
- (viii) Region
- (ix) Ecological zone

1. Sex : Male =1 Female =2
2. Age: ≤15 5 5 45 4
3. Educational Level
- No formal education =1
Secondary/SSS =3
Primary/JSS/Middle =2
others (specify) =4
4. Religion
- Christian =1
Traditionalist =3
Moslem =2
Others (specify) =4
5. Marital Status
- Married =1
Divorced =3
Widowed =5
Single =2
Separated =4
6. Main Occupation
- Farming =1
Fixed salary based job =3
Others (specify) =5
Hunting and gathering =2
Trading =4
7. Secondary Occupation

B. PRODUCTION

8. Production Levels of Bambara and other cereal legumes grown by the farmer

Type of legume	Acreage under cultivation	Yield/planting season or year	Proportion consumed	Proportion sold
Bambara				
Groundnut				
Soybean				
Cowpea				
Other, specify				

9. What are your reasons for growing Bambara?

Subsistence =1

For cash =2

For both =3

Other (specify) =4

10. Does Bambara give you more income than other legumes grown?

Yes =1

No =2

11. If no to Q10, which legumes give you a higher income?

Groundnut =1

Soybean =2

Cowpea =3

Other (specify) =4

12. In which months of the year is Bambara in high supply?.....

13. In which months of the year is Bambara in low supply?.....

14. Has your production of Bambara increased or decreased over the past 3years?

Increased =1

Decreased =2

Same =3

Don't know =4

15. What counted for the increase in the production if production has increased?

Good Weather =1

Increased capital =2

Higher income =3

Acquisition of bigger land =4

Other (specify) =5

N/A =6

16. What accounted for the decrease in the production if production has decreased?

Poor Weather =1

Lack of capital =2

Reduced income =3

Lack of land =4

Other (specify) =5

N/A =6

17. What is the first (most important) constraint to Bambara production in the area?

Climate =1

Access to land =2

Labour =3

Inputs =4

Seed =5

Storage =6

Knowledge about production practices =7

Other (specify) =8

No constraint =9

18. What is the second constraint? 19. Land Tenure

Family/Own land =1

Rented =2

Leased =3

Shared cropping =4

Communal =5

Other (specify) =6

20. Indicate the type of labour and people involved in the various activities

Activity	Type of labour	Person(s) responsible
Land clearing		
Ploughing		
Harrowing		
Ridging		
Hoeing		
Planting		
Application of agro-chemical		
Harvesting		
Shelling		
Winnowing		
Storage		
Marketing		
Weeding		

Type of labour

Family =1 Hired =2 Combination (tick) =3 Other (specify) =4

Person(s) responsible

Husband =1 Wife =2 Both husband and wife =3 Male children =4
 Female children =5 All children =6 Every house hold member =7

21. Is labour readily available?

Yes =1 No =2 Sometimes =3

22. What is your source of seed?

Own seed =1
 From the market =3 From other farmers =2
 Other (specify) =5 From seed growers =4

23. Are the following easily accessible?

Yes =1 No =2

i. Extension services

ii. Irrigation infrastructure

iii. Mechanised services

24. Which agricultural machinery/equipment do you have access to? Tick those relevant

Cutlass =1 Hoes =2 Plough =3 Tractor =4 Planter =5
 Harvester =6 Others (specify) =7

25. Do you use any of the following for your Bambara production?

Yes =1 No =2

Fertilisers	
Insecticides on the field	
Insecticides for storage	
Weedicides	

Gender issues and responsibilities related to bambara production in the household

26. Please complete the table below by indicating with reasons which member of the family takes responsibility for the following activities:

Activity	Person(s) Responsible	Reasons
i. Preparation of land		
ii. Cultivation of Bambara		
iii. Harvesting of Bambara		
iv. Shelling of Bambara		
v. Storage of Bambara		
vi. Marketing of Bambara		
vii. Utilisation/Product development		

Husband =1 Wife =2 Both husband and wife =3 Male children =4
 Female children =5 All children =6 Every household member =7

C. STORAGE, PROCESSING AND UTILISATION

27. For how long do you dry Bambara after shelling before storage?
 <2days =1 2-4days =2 5-7days =3 >1week =4

28. How do you shell your Bambara after harvesting?
 Manually (specify) =1 Mechanised =2 Combination =3

29. How do you package the Bambara before storage?
 Jute sacks =1 Plastic sacks =2 Polyethylene bags =3
 Drums =4 Baskets =5 Bare floor =6 Other (specify) =7

30. Do you treat the Bambara with agro-chemicals before storage?
 Yes =1 No =2 Sometimes =3

31. If no to Q30, do you treat the Bambara by any traditional method before storage?
 Yes =1 No =2 N/A =3

32. Describe traditional pre-storage treatment if applicable

.....

33. Where do you store your Bambara?
 Storage barns =1 Rooms =2 Large basket =3 Other (specify) =4

34. Do you process Bambara into other products?
 Yes =1 No =2 Sometimes =3

35. If yes, how do you go about it?
 Mill into flour =1 Prepared into paste =2 Boiling =4
 Roasting =4 Combination (tick) =5 Other (specify) =6 N/A =7

36. How often is Bambara consumed in the household per week?
 Not often =1 1-3x =2 4-6x =3 Daily =4

37. What traditional meal is Bambara used for in the household?

47. What colour of Bambara groundnut do you prefer?

48. Why do you prefer this colour?

49. What grain size do you prefer?
 Big =1 Small =2 Medium =3

50. Why do you prefer this grain size?

E. MARKET OF BAMBARA GROUNDNUT

51. Where do you sell your Bambara groundnut?
 Open market =1 At home =2 Middle men =3 Supermarket =4
 Combination (tick) =5 Other (specify) =6 Do not sell =7

52. When do you sell your product?
 Immediately after harvest =1
 After storing for some time =2
 Both =3

53. Do you have problems with marketing of Bambara groundnut? If yes indicate the nature of the problem.
 Low demand =1 Low pricing =2 No problem =3 Other (specify) =4

54. Is there a special period for Bambara groundnut demand in your community?
 Yes =1 No =2 Sometimes =3

55. If yes to Q. 54, when is it?

56. How far do you travel to access a market for your Bambara groundnut crop?
 <5km =1 5-10km =2 11-15km =3 16-20km =4 >20km =5

57. Compared to other crops you grow, how do you rank Bambara groundnut marketing?
 Very difficult =1 Difficult =2 Very easy =3 Easy =4 Same =5

58. In which month do you have the highest pricing for Bambara and at what price do you sell a bag?

.....

59. In which month do you have the lowest pricing for Bambara and at what price do you sell a bag?

.....