Pre-breeding of Bambara Groundnut (*Vigna subterranea* [L.] Verdc.)

By

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Thesis Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is an under-utilized indigenous African legume crop which has substantial potential to contribute to food security in sub-Saharan Africa. The crop is well adapted to severe agro-ecologies and grows where other legumes may not survive. The seed is highly nutritious with an ideal balance of carbohydrate (55-72%), protein (18-20%) and fats (6-7% oil), which is particularly beneficial in balancing protein deficiencies in cereals. Also, the seed contains essential and non-essential amino acids of about 33% and 66%, respectively. These attributes make Bambara groundnut an ideal crop to alleviate food insecurity, and to reduce protein malnutrition in rural communities of Africa. However, small-scale farmers grow low-yielding landraces in most production regions in sub-Saharan Africa. Bambara groundnut landraces exist as heterogeneous mixtures of seeds of a few to several seed morpho-types that embrace wide genetic potential for breeding.

The objectives of this study were: 1) to determine the production status and constraints associated with Bambara groundnut production in Kano State of Nigeria through the use of a participatory rural appraisal (PRA); 2) to determine the genetic diversity of Bambara groundnut landraces through seed morphology; 3) to assess the inter- and intra-genetic diversity of the Bambara groundnut landraces; 4) to determine the yield and yield component responses among selected Bambara groundnut genotypes, 5) to determine the genomic diversity in Bambara groundnut landraces, using simple sequence repeat (SSR) markers; and 6) to develop a crossing protocol.

Using a structured questionnaire, 150 Bambara groundnut farmers were interviewed. The respondents interviewed were male and aged between 36 to 50 years, while Qur'anic education was the most popular among them. Most of the farmers practiced a combination of sole and mixed cropping, and allocated between 0.38 to 1.68 hectares of land to Bambara groundnut growing. They selected Bambara groundnut landraces, especially looking for large seeds that were pure and oval in shape, with a cream seed coat colour and which were early maturing. A total of 27 diverse landraces bearing different names were identified in the hands of the farmers. Most popular among them were Gurjiya, Kurasa, Hawayen-Zaki, Fara Mai-Bargo and Silva. Production was largely for home consumption and for sale on local markets. Common production constraints of the crop were identified as a lack of improved varieties (70.7%), frequent droughts (9.3%), low yield (4%) and limited access to large markets (3.3%).

Diverse collections of Bambara groundnut landraces from seven geographic origins were characterized using seed morphology, including seed coat, seed eye colour and pattern, and hilum colour and pattern. Out of 58 original seedlots, a total of 353 different seed morpho-types were further identified. The selected

morpho-types- can be used for large-scale production or true-to-type lines could be used in genetic improvement of the crop.

Genetic variability within- and between-landraces was investigated among 262 Bambara groundnut landraces, forty nine were studied for agronomic traits, and 213 were investigated for pod and seed variability. Most (47.9%) of the landraces developed pods with a point on one pole, and a round end on the other. Most had a creamy (37.1%) and yellow (76.1%) pod colour, and the pods were usually rough textured, and contained an oval seed. A further 158 landraces were evaluated for leaf morphology where 49.4% had round leaves, while 21.5% had elliptic leaves, with 55.7% of the landraces being heterogeneous, possessing more than one form of leaf shapes. These discrete characters can be utilized for genetic studies and improvement of Bambara groundnut.

Single plant selections of 49 Bambara groundnut genotypes were evaluated for yield and yield components using 26 yield and yield related traits. Highly significant variations ($P<0.001$) were detected among the genotypes for canopy spread, petiole length, weight of biomass, seed weight and seed height. Principal component analysis (PCA) identified nine useful components, where two components, PC_1 and PC_2, contributed strongly to the total variation, at 19% and 14%, respectively. The PCA revealed that leaf colour at emergence, petiole colour, leaf joint pigmentation and calyx colour were highly correlated with PC_1, while seed length, seed width and seed height had strong association with PC_2. Both the principal component and cluster analyses showed that most genotypes associated with one another with respect to agronomic and seed yield traits, irrespective of geographical location. The genotypes 211-57, MO9-4 and TV-27 displayed high seed yield performances, while TV-93 and 45-2 had higher biomass production. These genotypes can be used as breeding lines to enhance productivity of Bambara groundnut.

Fifty Bambara groundnut genotypes, representing seven geographical regions across Africa, were genotyped using five pre-selected polymorphic simple sequence repeat (SSR) markers developed specifically for Bambara groundnut. The results detected a total of 53 alleles among the 50 Bambara groundnut genotypes, while the neighbor-joining analysis generated seven major genetic groups, which were clustered regardless of their geographic origin. Close relationship were found between 211-68 on one hand and 211-83-2, N211K and M09-3 with 211-68 on the other. Genotypes M02-3, 211-55-1 and 211-57 displayed close similarities. These associations suggested the likelihood that the two pair groups had common origins or may possess similar genes.

A preliminary protocol was developed for crossing Bambara groundnut using eight selected parents, using the diallel mating system. Emasculation and crossing of Bambara groundnut was effective when conducted on the same day, with the two procedures being carried out sequentially between 4:30 am and

9:00 am. This protocol generated a number of F_1 seeds, with the most success being from crosses between 211-40-1 x N211-2, N212-8 x 211-40-1 and M09-3 x 211-82-1. These F_1 seeds can be advanced to confirm whether they are true F_1 or selfs.

The most important production constraint of Bambara groundnut production is the lack of improved varieties, suggesting that further breeding is needed to enhance productivity. Bambara groundnut landraces need to be sorted using discrete morphological features before breeding for genetic enhancement. The SSR markers used in the study demonstrated their ability to distinguish the existing diversity among the Bambara groundnut genotypes, which could be useful for both germplasm conservation and for breeding. Genotypes that displayed outstanding performance in seed yield and biomass can be used as breeding lines for the genetic improvement of Bambara groundnut. Overall, the study generated valuable and novel Bambara groundnut genetic material, useful in the development of improved cultivars for large-scale production in sub-Saharan Africa.

Declaration

I, Mohammed Sagir Mohammed, hereby declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research;
2. This thesis has not been submitted for any degree or examination at any other University;
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from those persons.
4. This thesis does not contain other authors' writing, unless specifically acknowledged as being sourced from other authors. 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;
    b) Where their exact words have been used, their writing has been replaced in italics and inside quotation marks and referenced;
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references section.

Signed: …… Date: ……

Mohammed Sagir Mohammed (Candidate)

As the candidate's supervisors, we agree to the submission of this thesis:

Signed: …… Date: ……

Prof. Hussein A. Shimelis (Principal Supervisor)

Signed: …… Date: ……

Prof. Mark D. Laing (Co-Supervisor)

Dedication

This thesis is dedicated to my late parents, Mal. Zakari Usman M. and Amina Zakari Usman who passed away and thus unable to witness the achievements I attained from their generous upbringing.
May their humble souls, rest in peace, amen.

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Thesis Introduction

The Bambara groundnut

The Bambara groundnut (*Vigna subterranea* [L.] Verdc.; **Syn:** *Voandzeia subterranea* [L.] Thouars.) is an under-utilized grain legume grown in Africa, mostly by women for food security (Ntundu *et al.*, 2006). Bambara groundnut is commonly referred as a poor man's crop. The crop is an important legume in Africa after cowpea (*Vigna unguiculata* [L.] Walp.) (Sellschop, 1962; Linnemann and Azam-Ali, 1993). Bambara groundnut has a high protein content (20%) (Rowland, 1993), which makes it a good complement for cereal-based diets in Africa. Bambara groundnut has the potential to improve nutrition, boost food security, foster rural development and support sustainable land use.

The center of origin of Bambara groundnut is believed to be 'Bambara', a place near Timbuktu in Central Mali, West Africa (Holm and Marloth, 1940). The suffix '-groundnut' is because of the way it sets its pods, which is similar to groundnut. Hence its common name is *'Bambara groundnut'*. The crop is now widely distributed and grown in Northern Australia, in Asia especially India, Indonesia, Malaysia, the Philippines and Thailand, New Caledonia, and in South America, particularly in Brazil (Rassel, 1960; Suwanprasert *et al.,* 2006). Important countries in West Africa producing Bambara groundnut include: Benin, Burkina Faso, Cote d'Ivoire, Gambia, Ghana, Guinea, Mali, Niger, Nigeria, Senegal and Togo in (Goli, 1997). In southern African, countries producing Bambara groundnut include Botswana, Madagascar, Malawi, Zambia, South Africa Swaziland, Tanzania and Zimbabwe. In the East and Central Africa, Burundi, Cameroon, Central African Republic, Congo, Ethiopia and Sudan produce substantial quantities of Bambara groundnut (Goli, 1997). Production of Bambara groundnut is limited to the semi-arid regions of Africa where rainfall is inconsistent and low, and water losses to run-off, drainage and evaporation may leave only a small proportion available for crop growth.

The seed of Bambara groundnut is consumed in several ways and at different stages of maturation, as a vegetable or snack. The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanut, and are made into a pudding (or steamed-paste) called Moi-Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzor *et al*., 2010). In Zambia, Bambara groundnut is used for bread making (Brough *et al*., 1993), and to produce legume milk (Poulter and Caygill, 2006). Dried seeds can be roasted and eaten as confectionery. The seed is regarded as a balanced food because when compared to most food legumes, it is rich in iron and its protein contains high level of lysine and methionine (Adu-Dapaah and Sangwan, 2004; Massawe *et al.,* 2005). Bambara groundnut contains approximately 20% protein, 63% carbohydrates and 18% oil. The fatty acid content is predominantly oleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). In a report by Suwanpraser *et al.* (2006), dried seeds were found to contain

18-20% protein, 55-72% carbohydrates and 6-7% oil, providing a balanced diet for humans. The variations in nutritional composition can be attributed to genotypic differences and genotype by environment interactions.

Rationale for pre-breeding and breeding of Bambara groundnut

For centuries, Bambara groundnut germplasm has been maintained as landraces, which are often phenotypically and genetically diverse. A landrace is a local variety of a plant species that evolved largely through selection by farmers in an unstructured way and which has becomes adapted to ecologies where it grows and survives (Nass and Paterniani, 2000). All cultivated Bambara groundnut genotypes are the result of unstructured mass selection from landraces that have evolved directly from their wild relatives, and which have adapted to harsh environments (Massawe *et al.,* 2005). Doku and Karikari (1971) reported that domesticated Bambara groundnut (*Vigna subterranea* var. *subterranea*) originated from its wild relative (*V. subterranea* var. *spontanea*) through a series of gradual natural and artificial selections that are still taking place. One example of such selection is a change from a spreading/trailing to a bunching growth habit, and reductions in leaflet area, shell thickness and days to flowering as a result of domestication. Landraces are popular among farmers for their yield stability under harsh environmental conditions (Doku and Karikari, 1971). The Bambara groundnut landraces can be systematically exploited in breeding programs through a dedicated **pre-breeding initiative.**

Pre-breeding refers to all concerted activities and/or procedures designed to identify desirable characteristics and/or heritable genes from otherwise un-adapted and unimproved plant genetic materials and their subsequent manipulation in the actual breeding of crop cultivars (Nass and Paterniani, 2000). It is a vital step that links conservation and the use of plant genetic resources especially for breeding. Pre-breeding enables precise and fast selection of suitable genetic sources and forms the initial steps of breeding. Pre-breeding is the route for genetic enhancement whose valuable agronomic characteristics can be used by plant breeders. How such activities are conducted, varies among breeders and crop species. Principal materials in pre-breeding exercise are the wild species and landraces because they harbor desirable genes necessary for improving yield, pest and disease resistance, food quality and adaptation. Nass and Paterniani (2000) defined pre-breeding activities to include the following: (1) the production of new base populations for a structured breeding program; (2) identify heterotic group for either hybrid production or further selection procedures; (3) the establishment of a core collection is possible only through pre-breeding when working with wild species and landraces. One of the key objectives of a core collection is to preserve a maximum level of genetic diversity in a minimum number of accessions. A core collection is dynamic in nature rather than a static set of accessions, which can be achieved through new introductions and/or replacements to meet changing breeding objectives (Nass and Paterniani, 2000).

Genetic diversity and crop improvement

Modern crop varieties have evolved from either genetically homogeneous (e.g. clones) or heterogeneous parents (e.g., seeds resulted from self- or cross fertilization) through careful selection and hybridization. These genetic resources are the basis for present and future food security. Despite its economic and nutritional values, Bambara groundnut is a little studied and under-utilized crop in sub-Saharan Africa. There is a lack of national and international research investment on indigenous crops with good nutritional qualities in favour of familiar crops of commercial interest, such as sugarcane, cocoa, coffee, cotton and groundnut (Massawe *et al*., 2005).

Thus far, the full genetic diversity of the crop remains largely unexploited in Africa. Hence, only farm level selection has been practiced wherein existing landraces are evaluated and their seeds multiplied for production (Massawe *et al*., 2005). There has been no targeted breeding of the crop and consequently there are no improved varieties of Bambara groundnut in the major growing areas of the African sub-region.

The International Cooperation with Developing Countries (INCO-DC) (http://www.wzw.tu-muenchen.de/pbpz/bambar/html/), including Botswana, Namibia and Swaziland, conducted a survey among 462 farmers and 115 consumers of Bambara groundnut during 2001 to 2003. The study reported farmers' preference of Bambara groundnut to include high yield, large pods, a spreading habit, early maturity and a short cooking time.

Low yields are common in this crop, which are often associated with poor seed germination and little or no fertilizer, leading to poor crop establishment in the dry regions (Linnemann and Azam-Ali, 1993). Reported yields were 649-1582 kg ha^{-1} in Swaziland with annual rainfall ranging between 633-728 mm. In Botswana, seed yield was only 68.5-159.9 kg ha^{-1}, where rainfall ranged between 389-433 mm yr^{-1}. However, the crop has the potential to produce yields up to 3 tons ha^{-1}, both in the field and in controlled environments (Collinson *et al*., 1996; 1999; 2000).

Genetic diversity within lines and populations is fundamental for breeding and germplasm conservation (Rana and Bhat, 2004; Murtaza *et al*., 2005). As such, knowledge of the genetic diversity among breeding materials is imperative to avoid the risk of increasing uniformity in elite germplasm, and in order to ensure long term selection gain. This is because crossing of a limited number of elite lines creates the danger of losing their genetic diversity.

Variability is principally achieved through conventional breeding. A conventional breeding program involves crosses followed by selection of superior recombinants from several segregating generations (Kumar, 1999). Furthermore, variability can be achieved through mutation breeding, which involves the

alteration of genetic composition of a genome using physical irradiation or chemical mutagens, to enhance heritable genetic variations for agronomic advantage. Such materials can further be used as inbred lines in advanced conventional breeding programmes.

Several marker-assisted breeding strategies are now available to plant breeders and geneticists that can be used to overcome some of the problems encountered during conventional breeding (Kumar, 1999). Marker assisted selection or marker aided selection (MAS) is a process whereby a marker (morphological, biochemical or DNA/RNA) is used for indirect selection of a genetic determinant of a trait of interest, such as yield, disease and insect resistance, abiotic stress tolerance, and/or a quality trait.

Information on the genetic diversity of Bambara groundnut landraces has been reported based on phenotypic features, especially agronomic traits (Ntundu *et al*., 2006) and seed traits (Olukolu *et al*., 2012), while those of molecular makers have been reported for within and between landrace diversity (Sambrook *et al*., 1989; Williams *et al*., 1990; Pasquet *et al*., 1999; Amadou *et al*., 2001; Massawe *et al*., 2002; 2003). Prominent among molecular markers used include Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) and diversity arrays technique (DArT) markers (Olukolu *et al*., 2012). Unfortunately, the dominance and rigid nature of the afore-mentioned marker systems makes them inappropriate for genetic diversity study and germplasm preparation and selection for genetic improvement (Somta *et al.*, 2011), particularly for Bambara groundnut. Simple sequence repeats (SSRs), also known as microsatellites, are found to be the makers of choice for diversity studies, including Bambara groundnut landraces (Lagercrantz *et al*., 1993). SSRs, which are short tandem repeats of DNA nucleotides, have the advantage of being multiallelic, co-dominant and evenly distributed throughout the genome of a species, and therefore easy to deploy when investigating pure line (self-pollinating crop) selection such as with Bambara groundnut landraces (Molosiwa *et al*., 2013). Being PCR based, SSRs are technically simple to deploy and are responsive to high throughput assays (Mansfield *et al*., 1994). They also have the advantage of being transferable among related crop species (Somta *et al.*, 2011). Somta *et al*. (2011) adopted SSRs from studies on adzuki bean, mungbean and cowpea, as well as those developed specifically for Bambara groundnut, for genetic analysis of Bambara groundnut landraces from different regions in Africa. In their study, they found great diversity among accessions from Africa, South-east Asia and those of unknown origin. Development and use of molecular markers in a marker-assisted selection programme, alongside genotypic and phenotypic characterization for diversity studies and mapping of agriculturally important traits of the available germplasm could assist in Bambara groundnut cultivar development. In this way, duplication of certain genotypes would be objectively avoided, which is particularly useful in genetic conservation and improvement programs. However, molecular markers

should not be seen as an alternative to the traditional crop improvement, but as tools to support conventional breeding.

Padulosi *et al*. (2002) reported that neglected and underutilized crops, including Bambara groundnut, might play a role in sustaining rural African populations by increasing their available food and protein uptake. From a research perspective, it is evident that the collection of Bambara groundnut germplasm held at the International Institute for Tropical Agriculture (IITA) has not been adequately characterized for use in breeding programs, relative to other legumes such as cowpea and soybean. Characterization of any available germplasm is a primary phase that helps a breeder to choose from several genotypes as starting point for long term improvement of the crop (Cilliers and Swanevelder, 2003). Consequently, the germplasm can be systematically studied using morphological traits (seed morpho-types) and molecular markers to identify unique germplasm for breeding. At this stage, such seed can further be studied by growing in preliminary field evaluations for assessment of their genetic worthiness. These procedures include some of the components of pre-breeding exercises that pave the way for the unbiased utilization of genetic resources of Bambara groundnut landraces.

The difficult nature of crossing Bambara groundnut has been widely reported (Goli, 1995; Kone *et al.*, 2007), probably due to the strict autogamous nature of the floral system of the crop (Onwubiko *et al.*, 2011). Breeders need a reliable, defined protocol to make crossing of selected parents. Improved varieties are yet to be developed and disseminated to boost productivity. Therefore, a pre-breeding program is a prerequisite to harness genetic diversity and identify potential parents for use in a Bambara groundnut breeding program.

Main Research Objective

The main objective of this research is to initiate pre-breeding of Bambara groundnut landraces from across Africa.

Specific objectives

1. To assess the production status and constraints associated with Bambara groundnut in the Kano State of Nigeria;
2. To determine the diversity of seed morphology of Bambara groundnut germplasm collections from seven different sources across Africa;
3. To determine the inter-and intra-morphological diversity of Bambara groundnut landraces collected from seven different sources;
4. To evaluate selected pure line of Bambara groundnut landraces for yield and important yield component traits;

5. To determine the genetic diversity of selected Bambara groundnut genotypes using SSR markers;
6. To optimize a protocol for the crossing of Bambara groundnut. By employing the protocol a diallel cross will be performed to determine heterosis and general and specific combining abilities of qualitative and quantitative characters among selected Bambara groundnut accessions.

Research hypotheses

1. Bambara groundnut production in Kano, Nigeria, is limited due to intermittent social and agronomic production constraints;
2. There is sizable variations for seed morphology among the Bambara groundnut landraces from the seven sources;
3. There is significant observable inter-and intra-morphological diversity among the Bambara groundnut landraces;
4. The Bambara groundnut landraces vary for yield and yield components;
5. SSR markers are capable of identifying genotypic differences among Bambara groundnut landraces, reflecting phenotypic variation;
6. Upon crossing of the Bambara groundnut landraces, their heterotic response, their GCA and SCA, in the segregating population can be accessed for superior traits especially seed yield, seed protein content, amino acid profile and other traits of agronomic interest.

The Thesis introduction is followed by Chapter One the literature review, and the research chapters which are distinct in accordance with a number of activities, related to the thesis objectives. Chapter Two to Six are written as discrete research papers intended for publication and may duplicate some aspects in other chapters. Some overlap and unavoidable repetition may exist between the chapters, especially with the references.

The referencing of this thesis follows the format of Crop Science, as per their "Instruction for Authors".

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CHAPTER ONE
A Review of the Literature

1.1 Introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is a legume crop that is an important food source for rural households in sub-Saharan Africa. It is well adapted to drought prone environments across the region. However, the productivity of Bambara groundnut is low due to limited breeding research and development in the past while it has the potential to produce up to 3 tons in both field and greenhouse conditions (Collinson *et al*., 1996). Also, the crop faces various stresses attributable to biotic, abiotic and socio-economic constraints. Unavailability of a dedicated pre-breeding and breeding program to supply improved, high yielding and locally adapted cultivars is considered to be the major constraint in the arid and semi-arid tropics preventing the full genetic exploitation of this crop.

Plant breeding involves two main activities, i.e., pre-breeding (plant-breeding research; germplasm enhancement) and cultivar development *per se*. These interdependent activities are the driving forces that determine the pace at which improved cultivars are released to farmers (Shimelis and Laing, 2012). Pre-breeding includes all activities directed at identification of desirable crop traits and/or genes and their transfer into a suitable set of parents for further selection. Pre-breeding involves the following activities: characterization of landrace populations; development of new parent populations to be used as breeding material with the long-term goal of using the best parents for cultivar development following progeny testing; introgression of new traits from other useful sources, usually a landrace or related species; creation of novel traits; acquisition of new information on crop genetics; and development of new plant breeding techniques. Therefore, the main focus of this study was to initiate a dedicated Bambara groundnut pre-breeding as the first step of breeding this valuable crop.

1.2 Bambara groundnut: taxonomy, origin and domestication

Bambara groundnut is an herbaceous, intermediate, annual (Fig. 1.1), self-pollinating crop belonging to the family Leguminosae, subfamily Papilionoideae and genus *Vigna* (Fatokun *et al*, 1993). The crop has its origin in Africa (Goli *et al.*, 1997). Both wild and cultivated species have $2n=2x=22$ number of chromosomes (Forni-Martins, 1986). The crop was called various names, including: Mandubi d' Angola (Marcgrav de Liebstad, 1648), while Linnaeus in 1763 designated it as *Plantarum*, and then re-named it *Glycine subterranea* (Goli *et al.*, 1997). In 1806, Du Petit-Thouars proposed the name *Voandzeia subterranea* [L.] Thouars. This name was popularly known and used by most researchers for a century. Botanical studies by Maréchal *et al*. (1978) revealed strong connections between Bambara groundnut and the genus *Vigna.* This was confirmed by Verdcourt (1980), who proposed a change of genus name to "*Vigna subterranea* [L.] Verdc." Also, Bambara groundnut has several common names such as, beans,

ground bean, earth pea and kaffir pea, depending on location and tradition. Common English names are Bambara groundnut, or Bambara. In Madagascar, it is called Madagascar groundnut, and in South Africa it is known as the Jugo bean while in Afrikaans it is Jugoboon (Kay, 1979; Tindall, 1983; Venter and Coertze, 1996). In Nigeria, it is called *Gurjiya* or *Kwaruru* (Hausa), *Ngamgala* (Kanuri), *Okpa* (Igbo), *Epa-kuta* (Yoruba) and *Kwam* (Goemai) Bambara groundnut (http://en.wikipedia.org/wiki/*Vigna_subterranea*). The Gha tribe in Ghana refers Bambara groundnut as *Akwei*. In Zambia it is called *Ntoyo* (ciBemba), *Ktoyo* (kiKaonde) and *Mbwiila* (chiTonga). In Swahili, it is *Njugumawe*, and *Voanjobory* (by French retailers, meaning, round peanut) (Hillocks *et al*., 2012). In Shangaan it is referred to *Tindluvu*; and in Shona and Ndebele (Zimbabwe) Bambara groundnut is called *Nyimo* and *Indlubu*, respectively.

The origin of Bambara groundnut has been debated for many decades. However, Rassel (1960), Hepper (1963) and Begemann (1988) all concurred that the crop has its origin in the African continent. Mali was considered to be the center of origin of Bambara groundnut because it was thought to be popular among a tribe called the Bambara, who live near Timbuktu in Central Mali, West Africa. However, the exact centre of origin of the crop in Africa remains unknown, because there is no evidence of spontaneous or wild forms of the crop in Mali. Dalziel (1937) reported the North of Yola province of Nigeria and near Garoua in northern Cameroon as centers of diversity. These findings were confirmed by Hepper (1963) and Begemann (1988). However, secondary centers of diversity exist outside Africa. Most of these countries are in Asia, including Sri-Lanka, Malaysia, Philippines and India, and Brazil (Rassel, 1960; Goli, 1997). In the case of South America, the crop's movement was associated with the era of slave trade. In South Africa, it has been speculated that Bambara groundnut was introduced to Southern KwaZulu-Natal by immigrants from North Africa (Swanevelder, 1998). In South Africa, production is limited mostly to northern part of Mpumalanga and KwaZulu-Natal.

Botanical features of the crop have similarities with that of the groundnut (*Arachis hypogea* L.). The crop is an annual herbaceous plant bearing bunched leaves arising from creeping stems that grow close to the ground (Fig. 1.1) (Goli, 1997). The growth habit of the crop may be bunched (erect), semi-bunched or spreading. It is naturally self-pollinated (Basu *et al.* 2007). The leaves are trifoliate, forming a cluster arising from branched stems that are either purple or green (Figs. 1.2 and 1.3) in colour and are borne on a long, erect and glabrous petiole, thickened at the base. Stem branching begins early, about one week after germination (Goli, 1997). Up to 20 or more branches may be borne on a single plant, depending on the genotype. Stem colour may be pigmented green, or partial or wholly red (Goli, 1997). The plant has a well-developed tap root system (Fig. 1.3), with abundant lateral roots that grow geotropically (Massawe *et al*., 2002). The roots form nodules for nitrogen fixation, in association with suitable rhizobia especially

strains of *Bradyrhizobium* (Linnemann and Azam-Ali, 1993), which may be useful in intercropping and rotation system.

Fig. 1.1 Photo of Bambara groundnut at Ukulinga Research Farm, University of KwaZulu-Natal

Fig. 1.2 Bambara groundnut plant: green (left) versus purple (right) petiole pigmentation

Fig. 1.3 Bambara groundnut plant: Trifoliate leaves (left); and tap root system showing root nodules (right)

Two stipels subtend the terminal leaflets, which are assigned to each of the two lateral leaflets (Goli, 1997). The leaflets may be elliptic, lanceolate, round or oval (Fig. 1.4), and are attached to the rachis. The terminal leaflet is slightly larger than the lateral leaflets, with an average length of 6 cm and an average width of 3cm (Goli, 1997). Leaf veins may be pigmented red or whole green, while leaves may be light to dark green. Leaf and flower buds arise alternately at each node.

Fig. 1.4 Types of terminal leaf shapes in Bambara groundnut: oval (top left); lanceolate (top right; elliptic (bottom left); and round (bottom right)

The Bambara groundnut has papilionaceous flowers that stand on racemes that are attached to a long peduncle by the pedicel, alternately on stem nodes (Basu *et al*,. 2007). Papilionaceous flowers are those of the Leguminoseae or Fabaceae family, which have bilaterally symmetrical corolla, and have five petals that include a large upper petal (Standard) which encloses two lateral wings resembling a butterfly and a lower united keel petal (Basu *et al.,* 2007). Open flowers are mostly yellow in colour (Fig. 1.5), and occasionally white or red. Pedicels attain maximum length at the time of anthesis during which anthers dehisce. The stigma becomes receptive prior to opening of the flowers (Linnemann, 1994). Peduncles attain maximum length at initiation of pegging; and fertilization takes place the same day as anthesis. The interval between the openings of successive flowers in a raceme varies from 24 to 48 hours; that of flowers on the same peduncle does not exceed 24 hours, but flowers rarely open at the same time (Goli, 1997). New flowers open in the early hours of the morning and they are yellowish-white, but towards the evening, the colour changes from yellow to brown. Older flowers can be light brown (Goli, 1997). Flowers possess a pair of hairy epicalyces. The calyx consists of five hairy sepals, out of which four are formed on the upper and one on the lower sides of the flower, respectively (Goli, 1997). The former are usually jointed, while the latter is free and largely extended to form the keel. At anthesis, the standard petal unseals and extend out with a hollow at the tip that offers access to which ants may sporadically

enter both the unopened and open flowers (Doku and Karikari 1971a), and may cause out-crossing. The stamens are diadelphous. A diadelphous stamen is characterized by filaments that are united into two sets or groups. Nine out of ten have their filaments partly fused, with one isolated vexillary stamen (Goli, 1997; and Basu *et al.*, 2007). After a flower has been pollinated, and fertilization has occurred, the peduncle elongates to convey one or more ovaries to or just below the soil surface. Flowering in Bambara groundnut is thought to be day-neutral. However, continuous light has been shown to delay flowering by 6-11 days depending on genotype (Nishitani *et al*., 1988). Some pods of Bambara groundnut are formed just below or on the soil surface (Fig. 1.5), while that of groundnut are strictly formed below the soil surface (Linnemann, 1994). The developed pod of Bambara groundnut is a fruit; it attains its mature size within 30 days of fertilization, followed by seed development during the next 10 days.

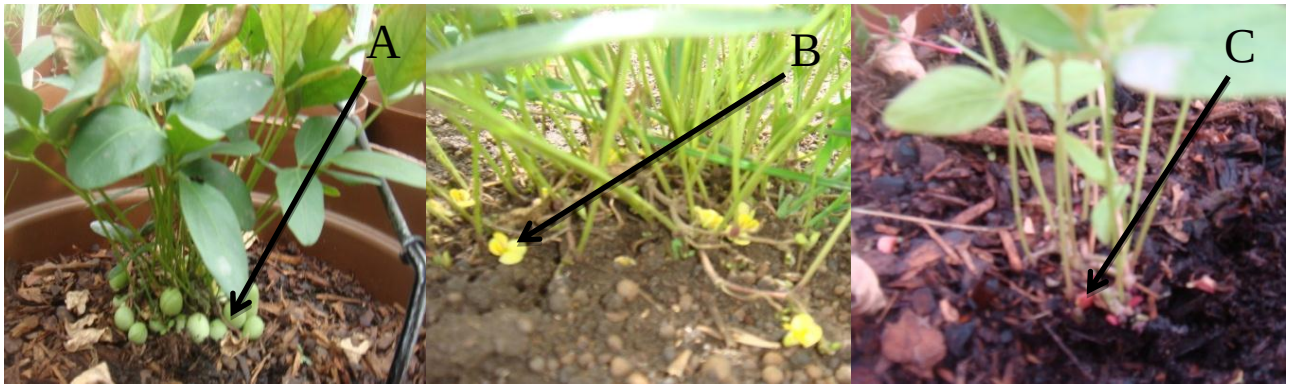

Fig. 1.5 A: development of pods above the ground level; **B** and **C**: Bambara groundnut landraces showing yellow and red flowers, respectively.

Goli (1997) reported that temperature may impact on the physiological maturity of pods in Bambara groundnut, with bunch types maturing earlier than spreading types. Linnemann and Azam-Ali (1993) evaluated the influence of photoperiod on fruit development, and found that a long photoperiod delays or even prevents fruit set in certain cultivars. In other words, there are photo- insensitive cultivars among Bambara groundnut landraces. Single-seeded pods are common in Bambara groundnut (Linnemann, 1994), but pods with three seeds have been reported in the Congo (Goli and Ng, 1988). Mature pods are indehiscent, often wrinkled, ranging from a yellowish or green, to a reddish dark brown or purple colour (Fig. 1.6). At maturity, seeds may vary in seed coat colour (white, cream, brown, dark brown, red, speckle and black); seed eye pattern (plain, black, red, brown, chalk-white and black- or red-butterfly) and size, and are usually smooth in texture and hard when dry (Stephens, 2003; Mohammed *et al*., 2013). The spreading types can be cross-pollinated, probably by ants during anthesis, while bunched types are almost entirely self-pollinating with the latter maturing earlier (Goli, 1997). Outcrossing in Bambara groundnut has been reported to vary among growing regions (Somta *et al*., 2011) with a minimum of 0% among

accessions from Tanzania and Thailand, and a maximum of 1.99% for West African accessions. A high level of outcrossing (4.99%) was observed in Guinea with a mean of 1.30%. However, the mean outcrossing is lower than that of other legumes such as mungbean (1.86%) and adzuki bean (3.52%) (Sangiri *et al*., 2007). Despite the variations in outcrossing Bambara groundnut flower remains cleistogamous. The period of anthesis has been found to reinforce low percent outcrossing in Bambara groundnut (Somta *et al.*, 2011).

Fig. 1.6 Yellowish pod (Top left); Dark purple pod (Top right); Light purple pod (Bottom left); and Green pod (Bottom right)

The Bambara groundnut is one of the most adaptable of all plants, tolerating harsh growing conditions better than most other crops. The crop is popularly grown in mixtures with other crops including cowpea, groundnut, maize and sorghum (Thottappilly and Rossel, 1997). It is ideally suited for hot, dry regions where growing other pulses is risky and unreliable. The crop is cultivated in ecologies up to 1600 m above sea level, with a mean temperature range of 20 to 28°C (Basu *et al*., 2007). It yields best in areas of low rainfall and does not yield well in times of heavy rainfall because it is drought tolerant does not shrives well on wetter soil conditions. Pod yields of 500-800 kg ha^{-1} are obtainable on poor soils, without any fertilizer application (Hillocks *et al*., 2012). The crop can grow and produce reasonable yield on laterite soils which are common in Africa (Mkandawire, 2007).

In sub-Saharan Africa, Bambara groundnut is mainly grown by female farmers (Ntundu *et al.*, 2004) as a mono-crop on a small scale. Research on Bambara groundnut has been limited compared with multidimensional studies made on sorghum, millet, maize, peanut and cowpea that are also popular in ecologies where Bambara groundnut is grown (Drabo *et al.*, 1997). Sérémé (1989), Sérémé *et al.* (1991) and Sérémé (1992) observed that little work has been reported regarding farming systems, conservation techniques and plant breeding of Bambara groundnut. Improving cultural and storage techniques, pest and disease control and using potential genetic resource for plant breeding could increase production and productivity of Bambara groundnut. Due to unavailability of improved cultivars in most growing areas, farmers grow landraces as the only available planting materials (Ofori *et al.*, 2006).

The planting date of Bambara groundnut varies between and among agro-ecologies. In southern Africa with a sub-Mediterranean climate, planting is usually in November/December and the harvest is made 5-6 months later (Hillocks *et al.*, 2012). In western Africa, planting is carried out in May-July and harvest in August/September with early plantings. Late planted crops are harvested in October/November. Bambara groundnut thrives best under bright sunshine, which is typical of the sub-Saharan climate which is favourable for its production (Linnemann and Azam-Ali, 1993; Directorate of Plant Production 2011). Growth and development to a mature crop generally takes between 3-5 or 6 months or 90-170 days after sowing (Linnemann and Azam-Ali, 1993), depending on the cultivar and time of planting. An annual rainfall of 300-600mm is sufficient for a successful crop on a well-drained soil (sandy to loam). At times, high temperatures complicate the crop's response to drought condition. With respect to such interactions, Shareef *et al.*, (2013) reported that both vegetative and reproductive growth may be affected by drought and temperature stresses, and that various Bambara groundnut cultivars may respond differently.

1.3 Economic importance of Bambara groundnut

Bambara groundnut is an African crop widely grown by subsistence farmers (Swanevelder, 1998). The seed is consumed in different ways and at different stages of maturity as a vegetable or snack. The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanut. The seed is made into a pudding (or steamed-paste) called Moi-Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzo *et al*. 2009). As a vegetable, the pods are sometimes harvested at the immature stage, boiled and eaten during the 'Hunger Period'. This is an interim period during the growing season when food stores are empty, but the main crops are not yet ready for harvesting. In Zambia, Bambara groundnut is used for bread making (Brough *et al.*, 1993), and to make legume milk (Poulter and Caygill, 2006). Dried seeds can be roasted and eaten as confectionery in the form of flat cakes and biscuits. Its flour can be mixed with cereals and made into porridge, as well as a component of infant feed. The seed provides a balance of carbohydrates, protein and fats, when compared to most high protein legumes which are used to

balance protein deficiencies in sorghum (*Sorghum bicolour* [L.] Moench) and maize (*Zea mays* L.) based diets (Adu-Dapaah and Sangwan, 2004; Massawe *et al.*, 2005). It is rich in iron, and the protein contains high lysine and methionine levels.

A few reports have been made on the medicinal benefits of Bambara groundnut. Leaves are used in Senegal to treat abscessed and infected wounds (Directorate Plant Production, 2011), while leaf sap is also applied to the eyes to treat epilepsy, and the roots are said to be useful as an aphrodisiac. Seeds can be pounded and mixed with water and taken for eye cataracts. In South Africa, raw seeds are chewed to cure nausea experienced by pregnant women (Directorate Plant Production, 2011). It is also a cheap source of vitamin B to prevent beriberi and is a superior source of vitamin B to many other legumes, including mungbean (*Vigna radiata* [L.] Wilczek) (Basu *et al*., 2007).

The seed of Bambara groundnut is highly nutritious and chemical analyses showed that it contains 32.7% of total essential amino acids and 66.1% non-essential amino acids (Minka and Bruneteau, 2000; Amarteifio *et al.,* 2006). Lysine is the major essential amino acid and accounts for 10.3% of the total essential amino acid in this crop. The seed of Bambara groundnut is also rich in leucine, histidine, valine and phenylalanine (Fetuga *et al*., 1975). The grain provides a complete balanced food (Rowland, 1993) making it a good supplement to cereal based diets such as sorghum, maize and millet. The seed contains approximately 20% protein, 63% carbohydrates and 18% oil. The fatty acid content is predominantly oleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). In another report by Suwanpraser *et al.* (2006), dried seeds were found to contain 18-20% protein, 55-72% carbohydrates, and 6-7% oil which is comparable with that of soybean (Poulter, 1981). Basu *et al*. (2007) showed that seed chemical composition comprise of 19.0% water, 3.4% ash, 22.2% crude protein and 6.6% oil, while carbohydrate and cellulose stood at 63.6% and 4.4%, respectively. Ferrao *et al.* (1987) found Bambara groundnut to be superior to groundnut in linoleic and palmitic acid content. It is also high in trypsin and chemotrypsin inhibitors (Aregheore, 1992). Processing Bambara groundnut seeds by roasting was found to greatly improve nutritional value by reducing the level of anti-nutritional factors (inhibitors). Roasting is widely practiced in Nigeria and roast seeds are eaten as a snack. Cooking time may impact the bioavailability of nutrients in Bambara groundnut seeds (Omoikhoje, 2008). Ijarotimi and Esho (2009) showed that fermentation improved mineral composition with minor effect on the amino acid profile. Furthermore, the procedure reduced the anti-nutritional factors present in the Bambara groundnut seed, including phytic and tannic acids, as well as oxalate and trypsin.

Olaleke *et al*. (2006) compared results of proximate analyses among legume grains including Bambara groundnut, cowpea (*Vigna unguiculata* [L.] Walpers), cranberry beans (*Phaseolus vulgaris*) and

Kersting's groundnut (*Macrotyloma geocarpum* [Harms] Marechal and Baudet). They found variation in constituents including moisture, ash, crude protein, crude fibre, carbohydrate and fatty acids.

1.4 Pre-breeding and Breeding of Bambara groundnut

1.4.1 Genetic diversity analysis using morphological and molecular makers

Bambara groundnut is primarily grown using landraces or farmers' varieties. . Farmers grow local landraces from previous harvests, or buy from local markets, because there are no available improved varieties of the crop for small or large scale production. This has been due to the lack of research on the crop towards its genetic enhancement.

Landraces are more phenotypically and genotypically diverse (Fig. 1.7) than pure lines, and are excellent sources of genetic variation for breeding (Zeven, 1998). Cultivated landraces were developed from the wild progenitor (*Vigna subterranea* var. *spontanea*) (Doku and Karikari, 1971b; Massawe *et al.*, 2005). Bambara groundnut is grown from landraces in all the major growing regions particularly in sub-Saharan Africa. Initial collections and evaluations of Bambara groundnut landraces were carried out by the International Institute of Tropical Agriculture (IITA) (Anonymous, 1947). Most national programs in Africa reportedly have multiple accessions of Bambara groundnut landraces in their germplasm collections (Goli, 1997). Some of these collections have been evaluated for diversity, multiplication or for agronomic research such as seed yield and plant population. For instance, the Institute for Agricultural Research, Samaru, Ahmadu Bello University in Nigeria has a mandate for the genetic improvement of the Bambara groundnut alongside other legumes including cowpea. Its scientists organized a second collection mission where about80 accessions were collected, multiplied and maintained. Promising lines were subjected to yield evaluation trials. Both morphological and yield characters were observed and recorded (Tanimu and Aliyu, 1990). The IITA in Ibadan, Nigeria has an international mandate for Bambara groundnut germplasm conservation, with over 2,000 accessions in stock, and there are over 1,000 accessions at the Office of Scientific and Technical Research Overseas (ORSTOM) in France. Other countries in Africa and Asia also have numerous Bambara groundnut accessions (Table 1.1).

Table 1.1 Countries/Institutions holding Bambara groundnut Germplasm collections ‡

Country/Institution	**Number of accessions held**
Benin	3
Botswana	26
Burkina Faso	143
France, ORSTOM	1000
Ghana, University of Ghana	80
Ghana, Savanna Agricultural Research Institute (SARI)	90
Ghana, Plant Genetic Resources Centre (PGRC)	166
Guinea	43
Kenya, National Genebank	6
Kenya, Kenya Agricultural Research Institute (KARI)	2
Kenya, National Museums	2
Mali	70
Mozambique	12
Namibia	23
Nigeria, IITA	2035
Nigeria	na
Niger	79
South Africa, Grain Crops Institute	198
South Africa, Institute for Veld and Forage Utilization	117
South Africa, Department of Agriculture	20
Tanzania, The National Plant Genetic Resources Centre of Tanzania (NPGRC)	22
Zambia, University of Zambia	463
Zambia, The National Plant Genetic Resources Centre (NPGRC)	124
Zimbabwe	129

‡Compiled from information provided by workshop participants, and the FAO Early Warning System on Plant Genetic Resources databases; n.a.= no data available; Source: (Adopted from Goli, 1997)

The Bambara groundnut germplasm held at IITA has not been adequately characterized for its use in breeding programmes especially relative to other legumes such as cowpea and groundnut. Padulosi *et al.* (2002) proposed that neglected and underutilized crops such as Bambara groundnut could play a prominent role in sustaining impoverished rural African populations by increasing their available food and protein uptake.

Fig. 1.7 Landraces of Bambara groundnut

Goli *et al.* (1997) characterized 1384 out of the more than 2000 accessions kept at IITA, and found significant genetic variation in growth habit and leaf shapes. Similar reports were made by Ntundu *et al.* (2006) on the morphological diversity among Bambara groundnut landraces in Tanzania. Ntundu *et al*. (2006) observed variation among Bambara groundnut landraces that revealed 63% being semi-bunch, 30% bunch and 7% spreading. Ofori *et al.* (2009) characterized Bambara groundnut landraces and observed variations in primary leaf colour of emerging seedlings to be 29% green and 71% purple. They observed that 89% of leaves were oval in shape, while 5.5% each were lanceolate and round. Number of days to flowering, pod length, and pod width presented low coefficients of variability when compared with number of leaves per plant, canopy spread and petiole length. Shelling percentage and shell thickness varied from 11.7 to 50.6% and 0.2 to 0.9 mm, respectively. In general, Ntundu *et al.* (2006) and Onwubiko *et al.* (2011) found that there was sufficient variation to breed Bambara groundnut. Ofori *et al.*, (2009) showed that variability among yield parameters may be related to variations among leaf shape, stem length, pod and seed production. Ofori *et al.* (2009), reported five groups in a principal component analysis of nine characters, with a minimum similarity of 40%, which corresponded to 58 different morpho-types out of the 70 accessions, representing 82% of these accessions. Pod colour was 57% yellowish, 37% brown and 6% reddish brown, while pod textures included smooth (14%), little grooved)77%), and much grooved (9%) (Ofori *et al.*, 2009). Qualitative traits were found to be significantly variable (Shegro *et al*., 2013), and therefore showed the importance of phenotypic markers for Bambara groundnut in genetic studies and improvement. (Olukolu *et al.* (2012) proposed the integration of qualitative and quantitative traits with molecular characterization in germplasm studies for pre-breeding. Maréchal *et al*. (1978) found variations between Bambara groundnut and other species of the genus *Vigna*

to which Bambara groundnut belongs, including cowpea (*Vigna unguiculata* [L.] Walp. Bambara groundnut differs with cowpea in that the latter bears its long pods above the ground on long robust stems. Distinctive morphological features of Bambara groundnut have been described by several authors (Doku and Karikari 1971b; Linnemann, 1994; Goli, 1997; Uguru and Ezeh, 1997; Basu *et al.*, 2007) (Table 1.2). Morphological descriptions used by previous studies were based on criteria defined by IPGRI (2000).

Molecular markers have been used in Bambara groundnut diversity studies (Pasquet *et al*., 1999; Amadou *et al*., 2001; Massawe *et al*., 2003; Singrün and Schenkel, 2004). However, simple sequence repeat (SSRs) DNA markers are found to be markers of choice for diversity analysis, particularly for Bambara groundnut landraces (Lagercrantz *et al*., 1993). They are short tandem repeats of DNA (Lagercrantz *et al*., 1993) which are multiallelic, co-dominant and evenly distributed throughout the genome of a species. They are useful markers to use when investigating pure line selections such as Bambara groundnut landraces (Molosiwa *et al*., 2013). Being PCR-based, SSRs are technically simple to deploy and are amenable to high throughput assays (Mansfield *et al*., 1994). In recent years, an important use of SSRs has been marker-assisted selection (MAS) in early generation breeding populations (Gupta and Varshney, 2000). Genetic characterization offers the capacity to detect genetic diversity that exceeds that of traditional (phenotypic) methods (de Vicente *et al*., 2005). DNA markers linked to agronomic traits can increase the efficiency of classical breeding by significantly reducing the number of backcross generations. Molecular markers should not, however, be seen as an alternative to the traditional characterization of cultivars by the use of morphological markers, rather they are supporting tools to such practices in conventional breeding.

Table 1.2 Some of the Bambara groundnut morphological descriptive characters and their variants used by previous studies

Morphological character	**Description**
Leaf colour at emergence	1 Green; 2 Purple
Terminal leaf shape	1 Round; 2 Oval; 3 Elliptic; 4 Lanceolate
Growth habit	1 Bunch; 2 Semi-bunch; 3 Spreading
Stem pigmentation	1 Whole green; 2 Light red; 3 Deep red
Petiole colour	1 Whole green; 2 Base purple; 3 Whole purple
Leaflet joint pigmentation of petiole	1 Green; 2 Purple
Pigmentation of flower wing	1 Present; 2 Absent
Open flower colour	1 Yellow; 2 White
Calyx colour	1 Green; 2 Purple
Fresh pod colour	1 Green; 2 Yellowish; 3 Light purple; 4 Deep purple
Dry pod colour	1 Yellow; 2 Brown; 3 Reddish-brown; 4 Purple
Pod shape	1 Without point; 2 Point-Round; 3 Point-Nook; 4 Point-Point
Pod texture	1 Smooth; 2 Little grooves; 3 Much grooves; 4 Much folded
Seed shape	1 Round; 2 Oval; 3 Ovate; 4 Spherical
Seed eye	1 Absent; 2 Present

Source: Adopted from observations made in this study

Abundant genetic resources of Bambara groundnut are maintained by various national research programs across Africa and IITA and other growing regions in the world. Genetic studies and targeted breeding of the crop are hampered possibly due to the difficulty of creating hybrids. As such, there is insufficient information on the successes of Bambara groundnut hybridization (Marandu and Ntundu, 1995; Kone *et al.*, 2007). The difficulty of emasculation and crossing in the crop limits even conventional crop-pollinations (Suwanprasert *et al*., 2006 and Onwubiko *et al.*, 2011). This is due to the small flower size and a lack of knowledge on its flower biology (Oyiga, 2010). Other reasons for the failure of the crop to set seeds after artificial crosses are limited pollen viability (Oyiga 2010), and development of flowers on or close to the ground level (Suwanprasert *et al*, 2006). Flowers and developing pods may be prone to diseases associated with rain or irrigation. The timing and methods of flower emasculation and pollination are fundamental issues in Bambara groundnut (Suwanprasert *et al.*, 2006).

Successful crosses have been reported between four distinct accessions of Bambara groundnut and the F_1 was advanced to the F_2 (Suwanprasert *et al*, 2006). INCO-DC (2002) in a BAMFOOD project reported

successful development of F_1 hybrids in crosses between domesticated Bambara groundnut landraces with wild *species* in Botswana and Swaziland (Massawe *et al*., 2003). However, there is no data available for confirmation and adoption of the technique used. Therefore, there is the need for a simple and affordable procedure that allows for effective hybridization in Bambara groundnut. Emasculations can be carried out by cutting the petal to expose the reproductive portion of the flower prior to pollen introduction; between 3:00pm and 10:00pm (Suwanprasert *et al*, 2006). Hybridization was found to be effective shortly after anthesis between 2:30am and 3:30am in Thailand. The need for a reliable protocol for hybridization of Bambara groundnut across growing ecologies is needed if speedy progress is to be made in the improvement of the crop.

Understanding the mode of inheritance of yield and yield components, and their association is basic for breeding. This aids choice of genotypes and breeding procedures for yield increase in crop species including Bambara groundnut. Genetic inheritance of yield and yield components were studied in cowpea, a related legume to Bambara groundnut by Aryeetey and Laing (1973). The study showed that most of the agronomic parameters were polygenic. Brittingham (1950) observed transgressive segregation for pod length and number of seed pods^{-1} in Bambara groundnut landraces. There was positive correlation between pairs of yield components (Aryeetey and Laing, 1973). There is strong relationship between 100 seed weight and shelling percentage, and that the former can be employed to select for high yield in Bambara groundnut.

Seed eye colour and pattern are variable traits in Bambara groundnut that may have breeding and agronomic values useful for cultivar selection. Seed eye pattern around the hilum is controlled by a single recessive gene (Oyiga *et al.,* 2010), who added that number of pods plant^{-1} and seed yield per plant had positively correlated. Oyiga *et al.* (2010) also described internode length as a measure of separating spreading from non-spreading growth habit. High heritability estimates were calculated by Ofori (1996) for number of leaves per plant leaf area and canopy spread, which can all be exploited through selection. Spreading genotypes have larger leaves and seeds, and exhibit indeterminate flowering habit. Threshing percentage varied from 11.7 to 50.6% (Ofori, 1996) among a number of Bambara groundnut landraces, with a pod coat thickness ranging from 0.2 to 0.9 mm. These traits may have implication with respect to insect pest, diseases and rodent activities that can be exploited in Bambara groundnut improvement. Ouedraogo *et al*. (2008) studied the phenotypic variability of Bambara groundnut accessions from northern Burkina Faso, and reported that plant canopy and number of pods per plant, seed width and seed length per plant as well as 100 seed weight were positively correlated with seed yield per plant. However, a negative correlation was observed between days to 50% flowering and yield plant^{-1}, meaning that longer vegetative growth could likely reduce yield in Bambara groundnut whereby more vegetative yield is

realized at the expense of seed yield. This study was limited because the collection of the landraces was only from one region (Goli *et al.*, 1997).

1.5 Yield potential and farmers' preferences of Bambara groundnut

World annual production of Bambara groundnut was about 330,000 tons (PROTA, 2006) with 45-50 % from West Africa. About one third of world annual production (10,000,000 kg) comes from Nigeria being the highest (Swanevelder, 1998), followed by Burkina-Faso with 44,000,000kgper annum. The crop has the potential of yielding >3000 kg ha^{-1} in both greenhouse and field trials (Collinson *et al*., 1996; 1999; 2000; Hillocks *et al*., 2012). However, performances vary under farmer management (Goli, 1997), probably due to prevailing agronomic conditions such as plant population, soil and genotype differences. Late planting was found to reduce seed yield drastically in Tanzania (Collinson *et al*., 2000). In Zimbabwe, yields range from 80-400 kg ha^{-1} under subsistence farmer management, high yields have been recorded with high plant density of 25,000 plants per ha using flat seed-bed and a semi-bunch landrace in Cote d' Ivoire (Kouassi and Zoro, 2010). Low seed yield in the crop was associated to poor seed germination which results to poor crop establishment in dry regions (Linnemann and Azam-Ali, 1993). In Swaziland, yields range from 649 and 1582kg ha^{-1} with rainfall ranging between 633-728mm per annum (Collinson *et al*., 2000). Conversely, in Botswana, seed yields of 68.5 and 159.9kg ha^{-1} were reported with rainfall ranging between 389 and 433mm yr^{-1} for the same period. Yield per plant has been measured 13.40 and 47.16g, with a mean of 28.89 g per plant (Nguy-Ntamag, 1997). Potential yield of the crop of >3000 kg ha^{-1} suggests that there is high yield can be exploited through breeding. Besides, farmer perception on characteristics associated with yield is limited probably because most of them grow the crop in intercropping with other companion crops, such as cereals, legumes and cassava.

Individual surveys related to farmers' perception and seed preferences were concurrently carried out in 2001 in Namibia, Swaziland and Botswana (Fleissner, 2001; Magagula *et al*., 2001; and Manthe *et al.*, 2001). Aggregated farmers' preferences for Bambara groundnut seeds are for early maturity, high yield, large pods, sweet taste, fast cooking, a spreading growth habit and cream-coloured.

1.6 Insect pests and diseases of Bambara groundnut

Pests, diseases and nematodes are the major yield limiting factors of Bambara groundnut (Thottappilly and Rossel, 1997).

1.6.1 Insect pests and nematodes

Few insect pests that have been reported to attack Bambara groundnut include groundnut leafhoppers (*Hilda patruelis* Stal), the larvae of *Diacrisia maculosa* L. and *Lamprosema indicate* Fabricius (Mabika and Mafongoya, 1997). *Piezotrachelus ugandum* L. and *Rivellia spp* were observed to cause damage on

developing pods and root nodules, respectively (Swanevelder, 1998). Termites have been found to attack pods in dry weather (Karikari *et al*., 1997). In West Africa, bruchids (*Callosobruchus maculatus* F. and *C. subinnotatus* Pic.) have been found to be the principal storage insect pests (Maina and Lale, 2004), but the latter is more damaging. Additionally, *C. maculatus* causes extensive damage on wide range of stored legume seeds (Drabo *et al.*, 1997; Maina and Lale, 2004). Damage by parasitic nematodes (*Meloidogyne incognita* [Kofoid & White] and *M. javanica* [Treub.]) on Bambara groundnut have been reported by researchers in Africa including Botswana (Karikari *et al*., 1997); Kenya (Ngugi, 1997); Zimbabwe (Mabika and Mafongoya, 1997) and South Africa (Swanevelder, 1998).

1.6.2 Viruses

Diseases play an important role in the productivity of Bambara groundnut. Drabo *et al*. (1997) reported loss of an entire germplasm collection due to foliar viruses in Burkina Faso. Viruses of Bambara groundnut have been reported in Nigeria (Thottappilly and Rossel, 1997) including cowpea aphid-borne mosaic virus, black-eye cowpea mosaic virus, peanut mottle potyvirus, cowpea mottle comovirus, and cowpea mosaic comovirus (cowpea yellow mosaic virus). Others are cowpea mild mottle carlavirus, cucumber mosaic cucumovirus and southern bean mosaic sobemovirus. The virulence of the aforementioned viruses to Bambara groundnut could be that the crop belongs to the same genus (*Vigna*) as cowpea. One or more of these viruses were earlier reported elsewhere (Robertson, 1966; Rossel, 1977; Shoyinka *et al*., 1978; Gumedzoe, 1985). Some of the principal vectors responsible for the spread of these viruses were aphids, whiteflies and beetles (Thottappilly and Rossel, 1997).

1.6.3 Fungi

Fusarium wilt has been found to be an important disease of Bambara groundnut in Kenya (Cook, 1978). Furthermore, rust and leaf blight, especially *Puccini* and *Colletotrichum* spp, respectively (Tanimu and Aliyu, 1997) have been reported to be prevalent in periods of high temperature and humidity in the Nigerian Guinea Savannah. Bambara groundnut sustains infection to leaf spot (*Cercospora canescens* Ellis & Martin), leaf blotch (*Phomopsis sp*.), powdery mildew (*Erysiphe sp*.) and *Sclerotium rolfsii* Sacc. (Gwekwerere, 1997). The presence of *S. rolfsii* (Sacc.) has been reported by Swanevelder (1998) in South Africa and late blight (*Corticium solani*) by Doku (1997) in Ghana. More important diseases are seed borne diseases mycoflora, particularly *Fusarium oxysporium* f. *sp*. *voandzeia* (Schleht.), *F. solani* (Mart.) Sacc., Michelia, *Aspergillus niger* (van Tiegh), and *A. flavus* (Link.) (Sérémé, 1989).

1.7 Conclusion

Bambara groundnut is an underutilized legume crop of African origin that has the potential of being a component of food security in sub-Saharan Africa. Seeds of the crop are consumed at different stages of growth and forms of utilization. The crop tolerates harsher environmental conditions better than most

other legumes. It is as good as other legumes in protein content (18-20%) (Minka and Bruneteau, 2000). Conversely, Bambara groundnut seed is superior in certain amino acids particularly lysine and methionine, which are important component of infant feed preparation. It is a complementary food to cereal based diets. Bambara groundnut is a member of the Leguminoseae family bearing Papilionaceous flowers similar to that of cowpea, wherein taxonomic and morphological features of the crop impede ease of artificial hybridization. However, in recent times successful hybridization of the crop has been reported (Suwanprasert *et al*., 2006; Oyiga, 2010).

Landrace collections of Bambara groundnut are being kept by both national and international programs and institutions within and outside Africa. The IITA, in Ibadan Nigeria has the international mandate for genetic resource conservation of Bambara groundnut. Unfortunately, the value of this under-utilized crop has not been adequately recognized. There are a number of reports on the characterization and evaluation of Bambara groundnut landraces using morphological and molecular markers in some countries in Africa (Goli, 1997). There is no detailed information on a dedicated Bambara groundnut breeding program with the subsequent release of improved varieties for farmers. Farmers are growing the crop below its potential level due to a lack of improved varieties.

Past and recent characterization studies have indicated high level of diversity among Bambara groundnut landraces that can be exploited through breeding. To exploit such potentials in the crop as source of desirable genes with agronomic benefits, there is a need for a pre-breeding program prior to the actual breeding of the crop for cultivar development (Shimelis and Laing, 2012). This will eventually open an avenue for a better exploitation of the genetic potential of Bambara groundnut.

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CHAPTER TWO

Production status and constraints of Bambara groundnut (*Vigna subterranea* [L.] Verdc.) in Kano State of Nigeria

Abstract

A baseline survey, using Participatory Rural Appraisal (PRA) was carried out among seven Local Government Areas (LGAs) in Kano State of Northern Nigeria to determine production status, farming practices, production constraints and perceived farmers' variety preferences of Bambara groundnut (*Vigna subterranea* [L.] Verdc.). Structured interviews through questionnaires were conducted using 150 Bambara groundnut farmers of 36 to 50 years of age. All respondents were male, married, and were growing Bambara groundnut. Qur'anic education is the most popular, representing 44% of the respondents whose Bambara groundnut farming practices were either sole and mixed cropping, and which dated back >20 years, using 0.38 to 1.68 hectares of land for Bambara groundnut production. All the farmers grow landraces, but the choice of landrace differed among farmers, with a greater preference for oval, large and pure seeds at 54.0%, 59.3% and 80.0%, respectively. Choices of cream seed coat colour and plants that mature early were also important. A total of 27 different Bambara groundnut landraces bearing different names were identified in the hands of the farmers. Production was largely for home consumption and local sale at local markets. Common production constraints to producing the crop include lack of improved varieties, frequent drought, low yields and poor market access. Incorporation of the framers' preferred characteristics into improved varieties would be a requirement for breeding goal of this crop to boost production and productivity of Bambara groundnut both for small and commercial production. This would also improve the livelihood, food security and income status of the growers as well as utilization, marketing and industrialization of the crop.

Keywords: Bambara groundnut, food security, landraces, Participatory Rural Appraisal, production constraints

2.1 Introduction

Grain legumes are the principal source of plant protein in tropical Africa among poor families (Massawe *et al*., 2005). Bambara groundnut (*Vigna subterranea* [L.] Verdcourt) is an under-utilized legume crop which originated in Africa and was cultivated long before groundnut (*Arachis hypogea* L.) (Goli *et al*., 1997). Like cowpea (*Vigna unguiculata* [L.] Walpers), another popular legume of African origin, Bambara groundnut is grown primarily for human consumption.

In addition to protein, the seed of Bambara groundnut is rich in carbohydrates and oils (Brough *et al*., 1993). Its content of essential and non-essential amino acids is 33% and 66%, respectively (Amarteifio *et al*., 2010). Bambara groundnut compared favorably with other legumes such as soybean in essential amino acids, namely lysine, methionine and cysteine (Fetuga *et al*., 1975). The crop is superior to mungbean (*Vigna radiata* [L.] Wilczek) in vitamin B (Basu *et al*., 2007).

Both fresh pods and dry seeds are used directly or processed to make different kinds of dishes. Fresh pods are boiled and eaten as snack, while dry seeds are processed into flour for the preparation of relish, such as 'Moi-moi' (a form of steamed paste), a traditional food made from soaked dry seeds, and puddled thereafter (Okpuzor *et al*., 2009). In paste form, the product is fried in oil and served with porridge at breakfast. The flour can be mixed with dry baobab leaves into paste, which is wrapped in maize leaves, and further steamed to make a traditional food, '*Tubani*'. Brough *et al*. (1993) and Massawe *et al*. (2005), and Fetuga *et al*. (1975) reported the use of Bambara groundnut seeds in making bread and vegetable milk, respectively. These attributes makes Bambara groundnut a valuable contributor to a balanced diet, thereby alleviating food insecurity, and making an important contribution to reducing protein malnutrition, which is common in rural communities in Africa (Ouedraogo *et al*., 2008; Shegro *et al*., 2013). Bambara groundnut seeds have also been used for the treatment of diarrhoea and stomach ache (Berchie *et al*., 2010).

The crop is tolerant to drought, and like other legumes, the roots develop nodules which possess the ability to fix atmospheric nitrogen through the activity of symbiotic soil bacteria (*Bradyrhizobium* species), thereby increasing the fertility level of the soil (Linnemann and Azam-Ali, 1993). This attribute may be useful in intercropping systems or sequential cropping especially with cereals.

However, the crop being under-utilized has not receive adequate research attention (Amadou *et al*., 2001) in contrast to other legumes including groundnut (*Arachis hypogea* L.), cowpea (*Vigna unguiculata* [L.] Walp.) and mungbean (*Vigna radiata* [L.] Wilczek) (Drabo *et al*., 1995).

Bambara groundnut is grown at a subsistence level with limited inputs (Massawe *et al*., 2005), mostly by women who usually intercrop it with cereals and other legumes, such as sorghum, millet, maize and

cowpea (DFID, 2002). The varieties grown are usually farmers' varieties or landraces, which comprise of various seed mixtures. Yields are usually low on farmers' fields, partly due to poor and variable seed germination (Linnemann and Azam-Ali, 1993) probably because the farmers are using seed mixtures of poor quality. Yields on farmers' fields in Botswana, Namibia and Swaziland have ranged from 649 to 1582 kg ha^{-1} (http://www.wzw.tu-muenchen.de/pbpz/bambara/html/). Baudoin and Mergeai (2001) reported yields between 300 and 800 kg ha^{-1} in Brussels, Belgium. At times yields can be unpredictable due to low inputs (Abu and Buah, 2011). However, Collinson *et al*. (2000) reported that yields > 3000 kg ha^{-1} can be obtained on research farms. The International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria has the mandate for Bambara groundnut germplasm conservation and research (Padulosi *et al*., 2002). Nevertheless, adequate diversity studies leading to genetic improvement and conservation of the available germplasm are still at infancy stage (Massawe *et al*., 2005; Olukolu *et al*., 2012). Yet Bambara groundnut remains a landrace, comprising of seed mixtures of various morpho-types whose seeds are planted, multiplied; and genetic diversity is commonly maintained by farmers for continuous use.

Currently, there are no improved and released varieties of the crop with better agronomic traits and seed quality for both small-scale and commercial production (Akpalu *et al*., 2013) when compred with other legumes such as cowpea and groundnut. The lack of any improved variety can result in a single landrace being maintained by Bambara groundnut farmers in different locations, with more than one name. As such, these landraces do not satisfy any agronomic, environmental or quality requirements by the Bambara groundnut growers. However, these landraces are genetic "treasures" that plant breeders need for the genetic improvement of the crop. Hence proper identification of such germplasm is imperative.

Crop varieties may be developed by breeders in research stations, where the breeders exclusively select the traits they bred for (Godfray *et al*., 2010), whereas the traits that farmers want may be given a lower priority less priority. Understanding farmers' needs and trait preferences should be a priority for plant breeders, if they want their new varieties to be adopted by a target audience of farmers. This can be effectively achieved through methods or approaches where farmers, who are the end users of any developed technologies, are adequately involved. This requires the design and development of an information collection system about the farmers and their choices. One such method is the participatory rural appraisal (PRA) approach.

Participatory rural appraisal was developed by Chambers (1992) to improve the understanding of values between scientists and farmers. The technique requires local knowledge to address the existing natural resources and agricultural systems, as well as health and socio-economic issues in societies needing prompt attention (Chambers, 1997; Loader and Amartya, 1999). Participatory rural appraisal has been

found to be useful by both government and non-governmental organizations in developing an understanding of social and infrastructural need and problems (Cornwall e*t al*., 2001). The use of PRA approach by Kafiriti (2004) was employed to understand farmers' abilities to diagnose and classify soil, to select rice varieties; and to track the exchange of information between farmers and researchers (Abera *et al*., 2013). Fashola *et al*. (2007) used a PRA approach to assess the adoption of maize varieties among farmers in Ethiopia**,** while Sibiya (2009) and Abakemal *et al*. (2013), and Olupot (2011) applied the technique to determine the most important constraints affecting sorghum and maize production, and to track varietal preferences in Uganda and KwaZulu-Natal, South Africa, respectively. There are a few PRA studies conducted to understand the production status, constraints and utilization of Bambara groundnut in most of its growing ecologies, such as those conducted by Berchie *et al*. (2010) and Akpalu *et al*. (2013) in Ghana, while Alhassan and Egbe (2013) conducted a similar PRA in Benue and Kogi States, Nigeria. There is a need for a well-structured survey using the PRA in order to discover the hidden problems and constraints affecting the production of Bambara groundnut in Kano State of Nigeria because Bambara groundnut is a locally important grain legume, along with groundnut and cowpea, despite the absence of any improved variety. Therefore, this study was conducted to determine production status, farming practices, production constraints and perceived farmers' variety preferences of Bambara groundnut (*Vigna subterranea* [L.] Verdcourt) using Participatory Rural Appraisal (PRA) among seven Local Government Areas (LGAs) in Kano State of Northern Nigeria.

2.2 Materials and methods

2.2.1 Study area and sampling procedure

A baseline survey was conducted among seven selected local government areas (LGAs) of Kano State, Northern Nigeria during 2012. Kano State is located at 12°37' N, 9°29' E and 7°43' W. Kano State is located in the Sudan Savannah zone, experiences a single maxima rainy season, which is between May/June to September/October each year, with a mean rainfall of 600 to 650 mm per annum. Mean temperature is between 30 to 35°C in the main (rain) season, and drops to 10 to 15°C in coolest dry season, which is between September and March each year. The entire geographic area of Kano falls in Sudan Savannah Zone and is characterized with environmental conditions with two seasons (dry and rainy), and similar rainfall pattern (an average of 690mm annum). Across the entire state, production of legumes is important, including Bambara groundnut.

Kano State comprise of three agricultural zones (i.e. Zone I, II and III) managed by the Kano State Agricultural and Rural Development Authority (KNARDA) with their administrative headquarters located in Rano, Dambatta and Gaya, in that order. In this study, two local government areas (LGAs) each from Zones I and III, and three LGAs from Zone II were purposefully selected based on their importance

to Bambara groundnut production in the State. The selected LGAs and their zonal headquarters from which the farmers were interviewed are listed in Table 2.1. For the successful conduct of the survey, farmers who grew Bambara groundnut were interviewed independently, after they had been identified with the assistance of Agricultural Extension Officers (AEOs) from KNARDA working in the respective LGAs. The AEOs also helped with the conduct of the interviews. Since the survey was carried out after the farmers had finished harvesting, house-to-house interviews were conducted. Pictures of Bambara groundnut seeds morpho-types with respect to seed coat colour and eye pattern were displayed to the farmers to aid perfection of farmers' responses at certain instances. Twenty questionnaires were issued in each LGA to twenty farmers, except for the Gwarzo LGA, where 30 questionnaires were issued to 30 farmers. A checklist of questions was designed to help as guide to obtain the desired information from the farmers, using 52 different variables. However, the farmers were also encouraged to provide their own views, to enhance the quality of information in the survey. A copy of the questionnaire was attached in appendix I.

Table 2.1 List of the Local Government Areas (LGAs) and their Zonal Headquarters used for the PRA

Zonal Headquarter	LGAs	Zonal Headquarter	LGAs	Zonal Headquarter	LGAs
Zone I (Rano)	Bebeji and Gwarzo	Zone II (Dambatta)	Bambatta, Dawakin-Tofa and Rimin-Gado	Zone III (Gaya)	Gabasawa and Gaya

2.2.2 Data analysis

Cross-tabulation was employed to perform chi-square analyses on discrete data. Analysis of variance (ANOVA) was performed on quantitative data using SPSS (SPSS, IBM Statistics 20) and Agrobase statistical packages (Agrobase, 2005; SPSS, 2011). In the ANOVA, treatment means were separated by the least significant differences (LSD) test at the 5% probability level.

2.3 Results and discussion

2.3.1 Age group and farming experience among Bambara groundnut farmers

The summary of chi-square tests on age group among the Bambara groundnut farmers in the seven selected LGAs in Kano State is presented in Table 2.2. There was a significant ($P< 0.05$) difference in age categories, where most of the farmers (60%) stood in the mid-age (36-50 years) group, and the largest contributor to this cohort was 17 farmers of this age group found in Gaya LGA. This indicated that both youths and the elders were involved in the production of Bambara groundnut in the selected LGAs of

Kano State. However, there were relatively more elderly farmers (>50 years) in Bebeji LGA, while in Rimin-Gado LGA none of the Bambara groundnut farmers was observed within the lower 25-35 years age group probably because most of the youth were involved in other businesses or were going to school especially tertiary institutions. Similar observation was made by Alhassan and Egbe (2013) who showed that most farmers in Benue and Kogi States in Nigeria were within the range of 41 to 50 years, whereas Abu and Buah (2011) found 97% of males and 3% of females growing Bambara groundnut were between the ages of 35 to 82 years. Scores on the number of years in farming occupation (Table 2.3) among the Bambara groundnut farmers did not show any variation, which indicated that farming is an unchanging occupation across the State.

Table 2.2 Summary of Chi-square tests on age group of Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Age in years of respondents			df	X^2	P-value	Number of valid cases
		25-35	36-50	>50				
Bebeji	Actual Count	1	9	10				
	Expected Count	1.7	12	6.3				20
Gwarzo	Actual Count	2	15	13				
	Expected Count	2.6	18	9.4				30
Dambatta	Actual Count	5	11	4				
	Expected Count	1.7	12	6.3				20
Dawakin-Tofa	Actual Count	2	15	3	12	29.352	0.03	
	Expected Count	1.7	12	6.3				20
Rimin-Gado	Actual Count	0	11	9				
	Expected Count	1.7	12	6.3				20
Gabasawa	Actual Count	0	12	8				
	Expected Count	1.7	12	6.3				20
Gaya	Actual Count	3	17	0				
	Expected Count	1.7	12	6.3				20

Legend: **df**=degrees of freedom

Table 2.3 Summary of Chi-square tests on number of years being a farmer among Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Number of years as a farmer (in years)					df	X^2	P-value	Number of valid cases
		<5	5-10	10-15	15-20	>20				
Bebeji	Actual Count	0	1	3	5	11				
	Expected Count	0.1	1.5	2.8	4.3	11.3				20
Gwarzo	Actual Count	0	0	3	6	21				
	Expected Count	0.2	2.2	4.2	6.4	17				30
Dambatta	Actual Count	0	2	3	1	14				
	Expected Count	0.1	1.5	2.8	4.3	11.3				20
Dawakin-Tofa	Actual Count	0	1	2	4	13	24	26.674	0.320	
	Expected Count	0.1	1.5	2.8	4.3	11.3				20
Rimin-Gado	Actual Count	0	2	4	3	11				
	Expected Count	0.1	1.5	2.8	4.3	11.3				20
Gabasawa	Actual Count	1	1	2	8	8				
	Expected Count	0.1	1.5	2.8	4.3	11.3				20
Gaya	Actual Count	0	4	4	5	7				
	Expected Count	0.1	1.5	2.8	4.3	11.3				20

Legend: **df**=degrees of freedom

2.3.2 Educational qualification of the Bambara groundnut farmers

There was a highly significant difference ($P < 0.001$) among the Bambara groundnut farmers interviewed in the study area on their educational qualification (Table 2.4). Most of the farmers (44.7%) had Qur'anic education to primary, secondary, tertiary and mass literacy education in that order, except for Dawakin-Tofa where most of the farmers had secondary school leaving certificates. Most of the Bambara groundnut farmers in Benue and Kogi States had benefitted from a modern education (Alhassan and Egbe, 2013), probably due their closer proximity to southern Nigeria where the modern education was introduced during the colonial era. None of the farmers in Bebeji, Gabasawa and Gaya LGs had educational qualification beyound secondary school. The result indicated a wide variation in level of education among the Bambara groundnut farmers. The popularity of Qura'anic education is in connection with the fact that Kano State is primarily dominated by Muslims where acquisition of Qur'anic education is mandatory to every Muslim. Also, while trading and agriculture remain the main occupations practiced by the indigenous people, western education came to the northern part of the country later than in southern Nigeria. Consequently, some farmers did not have the opportunity to acquire western education during their childhood.

2.3.3 Accessible sources of extension services of the Bambara groundnut farmers

Table 2.5 presents the available sources of extension services that were accessible to the Bambara groundnut farmers in the selected LGAs used in the study. Although the result did not show any significant difference, the data indicates the likelihood that there were real differences in the access to extension services among the Bambara groundnut farmers. Nonetheless, the validity of the result needs to be confirmed in future studies.The farmers generally have access to extension services rendered by a single government agency in the State, KNARDA. This authority was established in 1981 to provide a government-funded agricultural extension service in Kano State (KNARDA management, personal communication).

Table 2.4 Summary of Chi-square tests on educational qualifications of Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Education level of the respondents					df	X^2	P-value	Number of valid cases
		Qur'anic	Primary	Secondary	Tertiary	Mass literacy				
Bebeji	Actual Count	11	5	4	0	0				
	Expected Count	8.9	4.9	4.4	0.7	1.1				20
Gwarzo	Actual Count	13	9	7	0	1				
	Expected Count	13.4	7.4	6.6	1	1.6				30
Dambatta	Actual Count	9	5	5	1	0				
	Expected Count	8.9	4.9	4.4	0.7	1.1				20
Dawakin-Tofa	Actual Count	2	5	11	2	0	24	77.006	0.000	
	Expected Count	8.9	4.9	4.4	0.7	1.1				20
Rimin-Gado	Actual Count	7	3	1	2	7				
	Expected Count	8.9	4.9	4.4	0.7	1.1				20
Gabasawa	Actual Count	9	7	4	0	0				
	Expected Count	8.9	4.9	4.4	0.7	1.1				20
Gaya	Actual Count	16	3	1	0	0				
	Expected Count	8.9	4.9	4.4	0.7	1.1				20

Legend: **df**=degrees of freedom

Table 2.5 Summary of Chi-square tests on source of extension services accessible to Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Source of extension services					df	X^2	P-value	Number of valid cases
		Government	Mass media	Agricultural retailers	Neighboring farmers	Open markets				
Bebeji	Actual Count	15	2	1	0	2				
	Expected Count	14.8	1.2	2	0.7	1.3				20
Gwarzo	Actual Count	23	2	4	1	0				
	Expected Count	22.2	1.8	3	1	2				30
Dambatta	Actual Count	15	1	1	1	2				
	Expected Count	14.8	1.2	2	0.7	1.3				20
Dawakin-Tofa	Actual Count	13	0	3	3	1	24	30.384	0.172	
	Expected Count	14.8	1.2	2	0.7	1.3				20
Rimin-Gado	Actual Count	17	0	2	0	1				
	Expected Count	14.8	1.2	2	0.7	1.3				20
Gabasawa	Actual Count	18	1	0	0	1				
	Expected Count	14.8	1.2	2	0.7	1.3				20
Gaya	Actual Count	10	3	4	0	3				
	Expected Count	14.8	1.2	2	0.7	1.3				20

Legend: **df**=degrees of freedom

2.3.4 Inputs requirements and sources of crop inputs for the Bambara groundnut farmers in seven selected LGAs in Kano State, Nigeria

Farm inputs are basic requirements for any successful agricultural production. Results from Chi-square tests to most important inputs and their sources did not vary among the Bambara groundnut farmers (Tables 2.6 and 2.7), respectively. Although the result did not show any significant difference, the data suggests there is actually a difference which might be revealed in further study. Nonetheless, seed and fertilizer were observed to be most important for the farmers, with seed being the most important, while agricultural retailers remained the point of input supply to the respondents. Traditionally, farmers keep their own seed for next year's production because of the uncertainty of securing landraces that they are used to, and in the absence of any improved variety.

Table 2.6 Summary of Chi-square tests on most important input need by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Input acquisition		df	X^2	P-value	Number of valid cases
		Seed	Fertilizer				
Bebeji	Actual Count	20	0	6	9.964	0.138	20
	Expected Count	19.6	0.4				
Gwarzo	Actual Count	30	0				30
	Expected Count	29.4	0.6				
Dambatta	Actual Count	20	0				20
	Expected Count	19.6	0.4				
Dawakin-Tofa	Actual Count	19	1				20
	Expected Count	19.6	0.4				
Rimin-Gado	Actual Count	20	0				20
	Expected Count	19.6	0.4				
Gabasawa	Actual Count	18	2				20
	Expected Count	19.6	0.4				
Gaya	Actual Count	20	0				20
	Expected Count	19.6	0.4				

Legend: **df**=degrees of freedom

Table 2.7 Summary of Chi-square tests on source of inputs frequently accessed by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Source of inputs		df	X^2	P-value	Number of valid cases
		Government agency	Agricultural retailers				
Bebeji	Actual Count	8	12				
	Expected Count	8.1	11.9				20
Gwarzo	Actual Count	17	13				
	Expected Count	12.2	17.8				30
Dambatta	Actual Count	10	10				
	Expected Count	8.1	11.9				20
Dawakin-Tofa	Actual Count	4	16	6	8.399	0.210	
	Expected Count	8.1	11.9				20
Rimin-Gado	Actual Count	8	12				
	Expected Count	8.1	11.9				20
Gabasawa	Actual Count	6	14				
	Expected Count	8.1	11.9				20
Gaya	Actual Count	8	12				
	Expected Count	8.1	11.9				20

Legend: **df**=degrees of freedom

2.3.5 Cropping systems and practices employed by Bambara groundnut farmers

Farming systems practiced by the Bambara groundnut growers in the study were highly significantly different between respondents (P< 0.001) (Table 2.8). Sole cropping or mixed cropping were the key cropping systems practiced by the Bambara groundnut farmers. Mixed cropping was most popular among the LGAs except for Gwarzo where sole cropping was more important probably due to differences in cultural cropping systems across the study area. Alhassan and Egbe (2013) reported that 30% and 66% of farmers in Benue and Kogi States grew Bambara groundnut as sole crop or as an intercrop, respectively.

The culture of Bambara groundnut production revealed highly significant (P< 0.001) differences among the LGAs by the Bambara groundnut farmers (Table 2.9). Among the three identified cultures, pure seed and seed mixtures were more popular than intercropping Bambara groundnut with cereals. Most farmers in Dambatta, Dawakin-Tofa and Rimin-Gado (all in Zone II) practiced sole and pure seed culture. This was probably because they are located in similar agro-ecology, and partly due to the location of one of the big markets in Kano State, the Dawanau Agricultural Market sited in the Dawakin-Tofa LGA. The influence of the presence of agricultural stakeholders in and around the locality as well as farmer-to-farmer interaction may have played a role in orienting the farmers to the demands of customers and vendors for pure seeds. It was understood that even 'pure seed' practice was not pure in term of all

possible variations, due to variable seed coat colours, seed eye colours and hilum patterns. This means that the farmers' selection was not adequate for use in breeding program.

Table 2.8 Summary of Chi-square tests on Bambara groundnut cropping culture engaged by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Farming systems practiced by the respondents		df	X^2	P-value	Number of valid cases
		Sole cropping	Mixed cropping				
Bebeji	Actual Count	8	12				
	Expected Count	8.1	11.9				20
Gwarzo	Actual Count	17	13				
	Expected Count	12.2	17.8				30
Dambatta	Actual Count	10	10				
	Expected Count	8.1	11.9				20
Dawakin-Tofa	Actual Count	4	16	6	59.317	0.000	
	Expected Count	8.1	11.9				20
Rimin-Gado	Actual Count	8	12				
	Expected Count	8.1	11.9				20
Gabasawa	Actual Count	6	14				
	Expected Count	8.1	11.9				20
Gaya	Actual Count	8	12				
	Expected Count	8.1	11.9				20

Legend: **df**=degrees of freedom

Table 2.9 Summary of Chi-square tests on the culture Bambara of groundnut production among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Bambara groundnut production practice			df	X^2	P-value	Number of valid cases
		Pure seed sole	Sole seed mixtures	In crop mixture				
Bebeji	Actual Count	10	10	0				
	Expected Count	12.7	5.3	2				20
Gwarzo	Actual Count	25	5	0				
	Expected Count	19	8	3				30
Dambatta	Actual Count	15	5	0				
	Expected Count	12.7	5.3	2				20
Dawakin-Tofa	Actual Count	17	3	0				
	Expected Count	12.7	5.3	2	12	132.5	0.000	20
Rimin-Gado	Actual Count	18	2	0				
	Expected Count	12.7	5.3	2				20
Gabasawa	Actual Count	2	3	15				
	Expected Count	12.7	5.3	2				20
Gaya	Actual Count	8	12	0				
	Expected Count	12.7	5.3	2				20

Legend: **df**=degrees of freedom

2.3.6 Years of experience in Bambara groundnut production

Chi-square response on the assessment of Bambara groundnut production experience among the Bambara groundnut farmers from seven selected LGAs in the study area did not show any statistical variations (Table 2.10). This indicated that Bambara groundnut production is a stable farming practice among the farmers, and that the crop remains important with various uses. It was observed earlier that (60%) of the farmers were within the range 36 to 50 years with general farming experience; in addition, most of the farmers in the study area had > 20 years of experience growing Bambara groundnut.

2.3.7 Bambara groundnut production in companion with other food crops

Bambara groundnut is produced in mixtures with other crops or as a sole crop showing highly ($P< 0.001$) significant differences among growers (Table 2.11). Most farmers practiced sole cropping. On the other hand, farmers in Gabasawa and Gaya LGAs grow Bambara groundnut in mixture with sorghum. This may be attributed to differences in agro-ecological conditions, in that the two LGAs stood in the same Zone (Table 2.1). In Bambara groundnut mixed cropping cultures, sorghum is a more popular companion crop than millet and maize, probably because sorghum is most commonly used as staple food crop in Kano State. Most farmers in Benue and Kogi States grew Bambara groundnut intercropped with other crops (Alhassan and Egbe, 2013).

Table 2.10 Summary of Chi-square tests on number of years taken to Bambara groundnut production by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Number of years growing Bambara groundnut					df	X^2	P-value	Number of valid cases
		<5	5-10	10-15	15-20	>20				
Bebeji	Actual Count	3	4	3	1	9				
	Expected Count	1.6	4.1	4.1	4.1	6				20
Gwarzo	Actual Count	1	2	3	11	13				
	Expected Count	2.4	6.2	6.2	6.2	9				30
Dambatta	Actual Count	3	6	3	1	7				
	Expected Count	1.6	4.1	4.1	4.1	6				20
Dawakin-Tofa	Actual Count	2	7	4	4	3	24	32.709	0.110	
	Expected Count	1.6	4.1	4.1	4.1	6				20
Rimin-Gado	Actual Count	0	5	6	5	4				
	Expected Count	1.6	4.1	4.1	4.1	6				20
Gabasawa	Actual Count	1	3	5	5	6				
	Expected Count	1.6	4.1	4.1	4.1	6				20
Gaya	Actual Count	2	4	7	4	3				
	Expected Count	1.6	4.1	4.1	4.1	6				20

Legend: **df**=degrees of freedom

Table 2.11 Summary of Chi-square tests response on Bambara groundnut production in mixtures with other crops as practiced by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Companion crops of Bambara groundnut				df	X^2	P-value	Number of valid cases
		Sorghum	Millet	Maize	None				
Bebeji	Actual Count	7	3	0	10				
	Expected Count	6.5	1.3	0.4	11.7				20
Gwarzo	Actual Count	7	1	0	22				
	Expected Count	9.8	2	0.6	17.6				30
Dambatta	Actual Count	4	1	0	15				
	Expected Count	6.5	1.3	0.4	11.7				20
Dawakin-Tofa	Actual Count	6	0	0	14	18	52.756	0.000	
	Expected Count	6.5	1.3	0.4	11.7				20
Rimin-Gado	Actual Count	1	0	1	18				
	Expected Count	6.5	1.3	0.4	11.7				20
Gabasawa	Actual Count	15	2	2	1				
	Expected Count	6.5	1.3	0.4	11.7				20
Gaya	Actual Count	9	3	0	8				
	Expected Count	6.5	1.3	0.4	11.7				20

Legend: **df**=degrees of freedom

2.3.8 Bambara groundnut production in rotation with other crops

There was a highly significant difference ($P< 0.001$) among the Bambara groundnut farmers who practice Bambara groundnut rotation with other crops (Table 2.12). Most farmers at Bebeji, Gwarzo and Dawakin-Tofa LGAs rotated sorghum with Bambara groundnut, while most of the farmers from Gabasawa and Gaya do not rotate Bambara groundnut. In Dambatta LGA, millet is the most popular crop used in rotation by the Bambara groundnut farmers. Bambara groundnut rotation with rice was popular in the Bebeji and Gwarzo LGAs. Regional soil type and rainfall probably influenced the rotation cultures of the farmers. Bebeji and Gwarzo LGAs are important in rice production whereas millet is an important cereal in Dambatta LGA because of the soil type. Alhassan and Egbe (2013) showed that 30% and 66% of Bambara groundnut farmers in their study area grew the crop as the sole crop or in an intercrop with companion crops including cassava, groundnut and cowpea.

Table 2.12 Summary of Chi-square tests on Bambara groundnut production in rotation with other crops as practiced by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Rotation with other crops					df	X^2	P-value	Number of valid cases
		Sorghum	Millet	Maize	Rice	None				
Bebeji	Actual Count	11	1	0	5	3				
	Expected Count	8.3	3.5	0.9	0.7	6.7				20
Gwarzo	Actual Count	21	1	0	0	8				
	Expected Count	12.4	5.2	1.4	1	10				30
Dambatta	Actual Count	3	12	4	0	1				
	Expected Count	8.3	3.5	0.9	0.7	6.7				20
Dawakin-Tofa	Actual Count	15	5	0	0	0	24	138.856	0.000	
	Expected Count	8.3	3.5	0.9	0.7	6.7				20
Rimin-Gado	Actual Count	5	5	1	0	9				
	Expected Count	8.3	3.5	0.9	0.7	6.7				20
Gabasawa	Actual Count	0	2	2	0	16				
	Expected Count	8.3	3.5	0.9	0.7	6.7				20
Gaya	Actual Count	7	0	0	0	13				
	Expected Count	8.3	3.5	0.9	0.7	6.7				20

Legend: **df**=degrees of freedom

2.3.9 Source of Bambara groundnut seeds for planting, purposes for which Bambara groundnut is produced and methods of consumption

Chi-square tests on the source of planting material among the Bambara groundnut farmers were highly ($P< 0.001$) significant (Table 2.13). Most farmers used their own seeds, i.e., landraces which were recycled by the farmers from previous harvests. Almost 100% of the farmers in Gwarzo, Rimin-Gado and Gaya LGAs used their own seed. In Ghana, Berchie *et al*. (2010) reported that most farmers kept and used their own seed after harvest against next planting season.

Farmers' views on the purpose for which they produced Bambara groundnut were similar (Table 2.14). Most of the farmers produced the crop both for home consumption and to sell as a cash crop. Few farmers, 5% and 10% from Bebeji and Gwarzo LGAs, respectively produced the crop for medicinal reasons.

Variation on the forms of Bambara groundnut consumption was highly ($P< 0.001$) significant. Farmers in Bebeji, Gwarzo and Rimin-Gado LGAs consumed fresh pods more often than other forms (Table 2.15). Both fresh pods and dry pods and seeds were consumed in Dambatta, Dawakin-Tofa, Gabasawa and Gaya LGAs. Consumption of fresh pods is takes place when the pods are harvested before maturity, to be eaten as a vegetable. The crop matures when other crops are still in the field, a hunger period called "a time for brief hunger". Most farmers produced the crop for domestic consumption, with only a little of the crop being sold or given away as a gift to friends and relatives.

Table 2.13 Summary of Chi-square tests on source of Bambara groundnut seeds used by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Source of seed for planting			df	X^2	P-value	Number of valid cases
		Own seed	Open market	Retail shops				
Bebeji	Actual Count	15	0	5				
	Expected Count	14.8	1.2	4				20
Gwarzo	Actual Count	30	0	0				
	Expected Count	22.2	1.8	6				30
Dambatta	Actual Count	5	3	12				
	Expected Count	14.8	1.2	4				20
Dawakin-Tofa	Actual Count	18	2	0	12	84.491	0.000	
	Expected Count	14.8	1.2	4				20
Rimin-Gado	Actual Count	20	0	0				
	Expected Count	14.8	1.2	4				20
Gabasawa	Actual Count	4	4	12				
	Expected Count	14.8	1.2	4				20
Gaya	Actual Count	19	0	1				
	Expected Count	14.8	1.2	4				20

Legend: **df**=degrees of freedom

Table 2.14 Summary of Chi-square tests on the purpose of Bambara groundnut production by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

		Production purpose							
Local Government Areas	Class	Home consumption	Both home consumption and sale	Animal feed	Traditional medicine	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	0	18	1	1				
	Expected Count	0.7	16.5	2.3	0.5				20
Gwarzo	Actual Count	0	24	3	3				
	Expected Count	1	24.8	3.4	0.8				30
Dambatta	Actual Count	1	15	4	0				
	Expected Count	0.7	16.5	2.3	0.5				20
Dawakin-Tofa	Actual Count	1	17	2	0				
	Expected Count	0.7	16.5	2.3	0.5	18	18.405	0.429	20
Rimin-Gado	Actual Count	1	15	4	0				
	Expected Count	0.7	16.5	2.3	0.5				20
Gabasawa	Actual Count	1	19	0	0				
	Expected Count	0.7	16.5	2.3	0.5				20
Gaya	Actual Count	1	16	3	0				
	Expected Count	0.7	16.5	2.3	0.5				20

Legend: **df**=degrees of freedom

Table 2.15 Summary of Chi-square tests on the methods of Bambara groundnut consumption among Bambara groundnut farmers in seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Methods of consumption			df	X^2	P-value	Number of valid cases
		Fresh pods	Dry seeds	Both fresh pods and dry seeds				
Bebeji	Actual Count	10	5	5				
	Expected Count	7.7	4	8.3				20
Gwarzo	Actual Count	15	5	10				
	Expected Count	11.6	6	12.4				30
Dambatta	Actual Count	6	5	9				
	Expected Count	7.7	4	8.3				20
Dawakin-Tofa	Actual Count	3	4	13				
	Expected Count	7.7	4	8.3	12	35.0	0.000	20
Rimin-Gado	Actual Count	16	2	2				
	Expected Count	7.7	4	8.3				20
Gabasawa	Actual Count	1	5	14				
	Expected Count	7.7	4	8.3				20
Gaya	Actual Count	7	4	9				
	Expected Count	7.7	4	8.3				20

Legend: **df**=degrees of freedom

2.3.10 Disposal of Bambara groundnut and constraints associated with Bambara groundnut production in the study area

There was no variation among the Bambara groundnut farmers on the form of disposal of Bambara groundnut (Table 2.16). Both fresh and dry pods and seeds were sold on the market. However, fresh pods were frequently sold by growers in Bebeji, Gwarzo and Rimin-Gado LGAs. In Dawakin-Tofa and Gabasawa both fresh and dry pods and seeds were sold on the markets.

Farmers' constraints associated with Bambara groundnut production in the study area varied significantly ($P< 0.05$) (Table 2.17). Out of the nine identified constraints, lack of access to seed of improved varieties was considered to be the most important constraint. This was followed by drought, low yields and low market prices, in that order. Less important constraints were weeds, and leaf and pod pests and diseases. Farmers' views on these constraints could be due to lack of research attention (Ntundu *et al*., 2004) that would have led to the production of improved varieties which would solve most of the related limitations that hinder production and productivity of the crop. Drought was considered to be less important among the Bambara groundnut farmers in Benue and Kogi States of Nigeria (Alhassan and Egbe, 2013), probably because these two States fall within the southern Guinea Savannah that receives more rainfall than Kano State, which is often dry. Northern Nigeria falls in the Sudan Savannah zone, and receives less rainfall than Benue and Kogi State.

Table 2.16 Summary of Chi-square tests on the disposal of Bambara groundnut produced by Bambara groundnut farmers among seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Disposal of Bambara groundnut		df	X^2	P-value	Number of valid cases
		Fresh pods	Both fresh pods and dry seeds				
Bebeji	Actual Count	13	7				
	Expected Count	10.5	9.5				20
Gwarzo	Actual Count	19	11				
	Expected Count	15.8	14.2				30
Dambatta	Actual Count	11	9				
	Expected Count	10.5	9.5				20
Dawakin-Tofa	Actual Count	8	12				
	Expected Count	10.5	9.5	6	10.537	0.104	20
Rimin-Gado	Actual Count	12	8				
	Expected Count	10.5	9.5				20
Gabasawa	Actual Count	5	15				
	Expected Count	10.5	9.5				20
Gaya	Actual Count	11	9				
	Expected Count	10.5	9.5				20

Legend: **df**=degrees of freedom

Table 2.17 Summary of Chi-square tests on the constraints associated with Bambara groundnut production as experienced by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

		Constraints associated with Bambara groundnut production												
Local Government Areas	Class	Lack of improved variety	Poor Germination	Weeds	Leaf pests	Pod pests	Pod diseases	Drought	Poor pod yield	Low market price	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	17	0	0	0	0	0	1	2	0				20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				
Gwarzo	Actual Count	19	0	0	3	3	1	0	3	0				30
	Expected Count	21.2	1.2	0.2	1	0.6	0.2	2.8	1.2	1				
Dambatta	Actual Count	16	0	0	0	0	0	3	0	1				20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				
Dawakin-Tofa	Actual Count	13	5	0	0	0	0	1	1	0	54	58.2	0.002	20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				
Rimin-Gado	Actual Count	13	0	0	0	0	0	4	0	1				20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				
Gabasawa	Actual Count	17	0	0	1	0	0	1	0	1				20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				
Gaya	Actual Count	11	1	1	1	0	0	4	0	2				20
	Expected Count	14.1	0.8	0.1	0.7	0.4	0.1	1.9	0.8	0.7				

Legend: **df**=degrees of freedom

2.3.11 Choice of Bambara groundnut landraces based on pod colour and shape by Bambara groundnut farmers

Choice of Bambara groundnut landraces based on pod colour (Table 2.18) and shape (Table 2.19) among the Bambara groundnut farmers differed significantly ($P< 0.001$). Most farmers preferred creamy-yellow coloured pods. In Gwarzo and Gabasawa LGAs, brown and purple coloured pods, respectively, were also important. Pods without points on either ends (stem or flower ends) were preferred by all the Bambara groundnut farmers in the study area, except for in the Gaya LGA where most of the farmers showed no preference for any of the pod shapes. Choice of pods that had no point by most farmers could be related to ease of handling especially harvesting and threshing which were carried out manually.

Table 2.18 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on pod colour by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by pod colour					df	X^2	P-value	Number of valid cases
		Cream-Yellow	Brown	Reddish	Purple	No preference				
Bebeji	Actual Count	8	1	2	3	6				
	Expected Count	10.7	2.5	2.5	3.2	1.1				20
Gwarzo	Actual Count	13	5	4	8	0				
	Expected Count	16	3.8	3.8	4.8	1.6				30
Dambatta	Actual Count	19	1	0	0	0				
	Expected Count	10.7	2.5	2.5	3.2	1.1				20
Dawakin-Tofa	Actual Count	11	1	5	3	0				
	Expected Count	10.7	2.5	2.5	3.2	1.1	24	71.871	0.000	20
Rimin-Gado	Actual Count	5	3	5	6	1				
	Expected Count	10.7	2.5	2.5	3.2	1.1				20
Gabasawa	Actual Count	9	7	0	3	1				
	Expected Count	10.7	2.5	2.5	3.2	1.1				20
Gaya	Actual Count	15	1	3	1	0				
	Expected Count	10.7	2.5	2.5	3.2	1.1				20

Legend: **df**=degrees of freedom

Table 2.19 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on pod shape by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

		Choice of landraces by pod shape							
Local Government Areas	Class	No point	One point	Two points	No preference	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	12	3	2	3				
	Expected Count	9.5	3.9	0.7	6				20
Gwarzo	Actual Count	21	4	0	5				
	Expected Count	14.2	5.8	1	9				30
Dambatta	Actual Count	11	1	2	6				
	Expected Count	9.5	3.9	0.7	6				20
Dawakin-Tofa	Actual Count	9	5	1	5	18	58.231	0.000	
	Expected Count	9.5	3.9	0.7	6				20
Rimin-Gado	Actual Count	8	5	0	7				
	Expected Count	9.5	3.9	0.7	6				20
Gabasawa	Actual Count	9	9	0	2				
	Expected Count	9.5	3.9	0.7	6				20
Gaya	Actual Count	1	2	0	17				
	Expected Count	9.5	3.9	0.7	6				20

Legend: **df**=degrees of freedom

2.3.12 Choice of Bambara groundnut landraces, based on pod texture, seed shape and seed size by Bambara groundnut farmers

Choice of pod texture varied significantly (P< 0.001) among the Bambara groundnut farmers (Table 2.20). Amongst the seven LGAs in Kano State, most farmers preferred smoothed textured landraces. Some farmers considered that landraces with smooth pods are easier to harvest and thresh. Farmers in Gabasawa and Gaya LGAs showed preference for landraces with grooved textured pods, believing that these pods are less prone to attack by soil-borne and storage pests. This is probably because the two LGAs were from the same Zone and may have common culture and insect pests and disease problems. Few farmers from Bebeji and Gwarzo indicated no preference for pod texture. Preferences for particular pod textures may be associated with the culture and ecological condition under which the crop is produced.

The Bambara groundnut farmers' preferences on seed shape differed significantly (P<0.05) (Table 2.21). A majority of the farmers (54%) in the study area preferred oval to round seeds. Round shaped seeds were the choice of Bambara groundnut landraces among most farmers in Dawakin-Tofa and Rimin-Gado LGAs. Differences of choice based on seed shape may be related to mode of consumption pattern in the

localities, since the findings in this study showed that most farmers grow Bambara groundnut both for home consumption and to sell their surplus.

Distribution of Bambara groundnut farmers on seed size preference did not vary in the study area (Table 2.22). However, large seeded landraces were preferred my most of the farmers in all the seven LGAs studied. This could be related to preferences for home utilization and how large seeds appeal to vendors in the markets. However, research based assessments measure seed size in terms of the 100 seed weight (g). Typically, large, medium and small seed have 100-seed weight measures of >120g, 70 to <100g and <70g, respectively (Ouedraogo *et al*., 2008; Berchie *et al*., 2010; Jonah *et a*l., 2010).

Table 2.20 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on pod texture by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by pod texture				df	X^2	P-value	Number of valid cases
		Smooth	Grooved	Folded	No preference				
Bebeji	Actual Count	9	7	1	3				
	Expected Count	11.2	6	1.9	0.9				20
Gwarzo	Actual Count	14	2	10	4				
	Expected Count	16.8	9	2.8	1.4				30
Dambatta	Actual Count	16	4	0	0				
	Expected Count	11.2	6	1.9	0.9				20
Dawakin-Tofa	Actual Count	18	1	1	0	18	80.718	0.000	
	Expected Count	11.2	6	1.9	0.9				20
Rimin-Gado	Actual Count	14	4	2	0				
	Expected Count	11.2	6	1.9	0.9				20
Gabasawa	Actual Count	6	14	0	0				
	Expected Count	11.2	6	1.9	0.9				20
Gaya	Actual Count	7	13	0	0				
	Expected Count	11.2	6	1.9	0.9				20

Legend: **df**=degrees of freedom

Table 2.21 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on seed shape by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by seed shape			df	X^2	P-value	Number of valid cases
		Round	Oval	No preference				
Bebeji	Actual Count	7	13	0				
	Expected Count	8.9	10.8	0.3				20
Gwarzo	Actual Count	9	19	2				
	Expected Count	13.4	16.2	0.4				30
Dambatta	Actual Count	10	10	0				
	Expected Count	8.9	10.8	0.3				20
Dawakin-Tofa	Actual Count	15	5	0	12	26.637	0.009	
	Expected Count	8.9	10.8	0.3				20
Rimin-Gado	Actual Count	13	7	0				
	Expected Count	8.9	10.8	0.3				20
Gabasawa	Actual Count	9	11	0				
	Expected Count	8.9	10.8	0.3				20
Gaya	Actual Count	4	16	0				
	Expected Count	8.9	10.8	0.3				20

Legend: **df**=degrees of freedom

Table 2.22 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on seed size by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by seed size				df	X^2	P-value	Number of valid cases
		Small	Medium	Large	No preference				
Bebeji	Actual Count	3	6	11	0				
	Expected Count	2.1	5.2	11.9	0.8				20
Gwarzo	Actual Count	4	6	16	4				
	Expected Count	3.2	7.8	17.8	1.2				30
Dambatta	Actual Count	1	7	12	0				
	Expected Count	2.1	5.2	11.9	0.8				20
Dawakin-Tofa	Actual Count	4	4	12	0	18	23.065	0.188	
	Expected Count	2.1	5.2	11.9	0.8				20
Rimin-Gado	Actual Count	2	4	14	0				
	Expected Count	2.1	5.2	11.9	0.8				20
Gabasawa	Actual Count	1	9	10	0				
	Expected Count	2.1	5.2	11.9	0.8				20
Gaya	Actual Count	1	3	14	2				
	Expected Count	2.1	5.2	11.9	0.8				20

Legend: **df**=degrees of freedom

2.3.13 Choice of Bambara groundnut landraces based on seed feature and seed coat colour by Bambara groundnut farmers

Selection of Bambara groundnut landraces based on seed features among the respondents differed significantly higher (P< 0.001) (Table 2.23). It appeared that the farmers preferred pure seed than seed mixtures. But a small number of farmers had no choice of any seed feature for production.

Bambara groundnut farmers' preference with respect to seed coat colour was significantly different (P< 0.001) (Table 2.24). Most of the farmers (65.3%) in the study area choose to grow cream coat coloured seed to black eye colour, followed by cream seeds with red eye. In Gabasawa LGA, the farmers preferred brown coat coloured seeds, followed by cream seeds with a black eye, in seed mixtures. Seed mixtures were not popular in most regions, as observed above, which may be related to consumption culture that lighter coloured seeds may be more appealing to the eyes. Berchie *et al*. (2010) found farmers preferred seeds that were white and large. Such choices of specific traits by farmers have research implications so that the breeders should breed for varieties that meet the requirements of the farmers.

Table 2.23 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on seed feature by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

		Choice of landraces by seed feature						
Local Government Areas	Class	Pure seed	Seed mixture	No preference	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	18	2	0	12	29.079	0.004	
	Expected Count	16.1	2.9	0.9				20
Gwarzo	Actual Count	22	4	4				
	Expected Count	24.2	4.4	1.4				30
Dambatta	Actual Count	17	1	2				
	Expected Count	16.1	2.9	0.9				20
Dawakin-Tofa	Actual Count	17	3	0				
	Expected Count	16.1	2.9	0.9				20
Rimin-Gado	Actual Count	19	1	0				
	Expected Count	16.1	2.9	0.9				20
Gabasawa	Actual Count	10	9	1				
	Expected Count	16.1	2.9	0.9				20
Gaya	Actual Count	18	2	0				
	Expected Count	16.1	2.9	0.9				20

Legend: **df**=degrees of freedom

Table 2.24 Summary of Chi-square tests on the choice of Bambara groundnut landraces based on seed coat and eye colour by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landrace by seed coat and eye colour							df	X^2	P-value	Number of valid cases
		Cream black eye	Cream red eye	Brown seed coat	Speckle seed coat	Red seed coat	Seed mixture	No preference				
Bebeji	Actual Count	16	1	0	3	0	0	0				
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20
Gwarzo	Actual Count	20	5	5	0	0	0	0				
	Expected Count	19.6	3	4.4	2	0.2	0.6	0.2				30
Dambatta	Actual Count	15	3	0	2	0	0	0				
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20
Dawakin-Tofa	Actual Count	17	0	0	3	0	0	0	36	74.056	0.000	
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20
Rimin-Gado	Actual Count	10	4	5	1	0	0	0				
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20
Gabasawa	Actual Count	6	1	8	0	1	3	1				
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20
Gaya	Actual Count	14	1	4	1	0	0	0				
	Expected Count	13.1	2	2.9	1.3	0.1	0.4	0.1				20

Legend: **df**=degrees of freedom

2.3.14 Choice of Bambara groundnut landraces based on growth habit, maturity and seed quality traits by Bambara groundnut farmers

There were highly ($P< 0.001$) significant differences among respondents on the choice of landraces based on growth habit (Table 2.25). The Bambara groundnut farmers preferred landraces with erect (bunch) habit, followed by semi-erect types. In Gabasawa LGA, the farmers indicated interest in landraces with a spreading habit. Few farmers in Gwarzo and Gaya LGAs showed no choice of any landrace with respect to growth habit.

There was a highly significant variation ($P< 0.001$) among the Bambara groundnut farmers in their preference for maturity period (Table 2.26). All farmers in the study area indicated that they preferred early maturing landraces to medium and late maturing types, except for Gabasawa who preferred medium maturing landraces. Growth habit and maturity seem to be related in respect of the farmers' selection of erect landraces which have the tendency to mature early. Further, farmers' preference for early maturity may be associated with the need for some food in times when other crops are still in the field.

Farmers' preference for seed quality (taste and cooking time) differed significantly ($P< 0.05$) (Table 2.27). Good taste was preferred than cooked time. Some farmers from Gwarzo, Rimin-Gado and Gabasawa LGAs showed no preference. It was observed that the Bambara groundnut farmers grow the crop for both home consumption and for sale, and that most farmers consumed some fresh pods. It is suggested that these habits may impact farmers' preference for good taste than fast cooking. Abu and Buah (2011) observed in their study that Bambara groundnut farmers dislike seeds that require a longer cooking period, and this may have breeding implication.

Table 2.25 Summary of Chi-square tests on the choice of landraces based on growth habit by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by growth habit				df	X^2	P-value	Number of valid cases
		Erect (Bunchy)	Semi-erect	Spreading	No preference				
Bebeji	Actual Count	20	0	0	0				
	Expected Count	11.3	3.6	4.4	0.7				20
Gwarzo	Actual Count	14	9	7	0				
	Expected Count	17	5.4	6.6	1				30
Dambatta	Actual Count	11	0	7	2				
	Expected Count	11.3	3.6	4.4	0.7				20
Dawakin-Tofa	Actual Count	11	4	5	0				
	Expected Count	11.3	3.6	4.4	0.7	18	71.418	0.000	20
Rimin-Gado	Actual Count	13	3	4	0				
	Expected Count	11.3	3.6	4.4	0.7				20
Gabasawa	Actual Count	1	11	8	0				
	Expected Count	11.3	3.6	4.4	0.7				20
Gaya	Actual Count	15	0	2	3				
	Expected Count	11.3	3.6	4.4	0.7				20

Legend: **df**=degrees of freedom

Table 2.26 Chi-square response on the choice of landraces based on maturity by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

		Choice of landraces by maturity						
Local Government Areas	Class	Early maturing	Medium maturing	Late maturing	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	11	3	6	12	75.025	0.000	
	Expected Count	13.2	4.4	2.4				20
Gwarzo	Actual Count	26	2	2				
	Expected Count	19.8	6.6	3.6				30
Dambatta	Actual Count	12	3	5				
	Expected Count	13.2	4.4	2.4				20
Dawakin-Tofa	Actual Count	14	6	0				
	Expected Count	13.2	4.4	2.4				20
Rimin-Gado	Actual Count	14	2	4				
	Expected Count	13.2	4.4	2.4				20
Gabasawa	Actual Count	3	17	0				
	Expected Count	13.2	4.4	2.4				20
Gaya	Actual Count	19	0	1				
	Expected Count	13.2	4.4	2.4				20

Legend: **df**=degrees of freedom

Table 2.27 Summary of Chi-square tests on the choice of landraces based on seed quality traits by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Choice of landraces by seed quality: Taste	Fast cooking	No preference	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	20	0	0				
	Expected Count	16.7	2.1	1.2				20
Gwarzo	Actual Count	27	0	3				
	Expected Count	25	3.2	1.8				30
Dambatta	Actual Count	17	3	0				
	Expected Count	16.7	2.1	1.2				20
Dawakin-Tofa	Actual Count	13	7	0	12	31.31	0.002	
	Expected Count	16.7	2.1	1.2				20
Rimin-Gado	Actual Count	14	3	3				
	Expected Count	16.7	2.1	1.2				20
Gabasawa	Actual Count	15	2	3				
	Expected Count	16.7	2.1	1.2				20
Gaya	Actual Count	19	1	0				
	Expected Count	16.7	2.1	1.2				20

Legend: **df**=degrees of freedom

2.3.15 Commonly grown Bambara groundnut landraces

Twenty four common names of Bambara groundnut landraces frequently grown by the Bambara groundnut farmers in the study area (Table 2.28). Production of the common landraces among the farmers differed significantly ($P< 0.001$). The most popular landrace was Gurjiya from Gabasawa LGA as indicated by 12 Bambara groundnut farmers, followed by Kurasa in Dambatta LGA (11 farmers). Gurjiya was also important in Dambatta LGA among 7 farmers. The popularity of Gurjiya is expected, because irrespective of the common name that any farmer, consumer or vendor may call it, Bambara groundnut is commonly called '*Gurjiya*' in Nigeria, particularly in the northern region including Kano State. However, other landraces were only represented in only one LGA throughout the study area. Most of these local names were associated with seed colour and source. Ten common names of landraces were identified between two States of Benue and Kogi among six communities (Alhassan and Egbe, 2013). The names may be related to culture, agronomic behaviour, and growth habits or seed characteristics such as colour or size. Akpalu *et al*. (2013) found four different landraces that the farmers grew in one community. It is probable some landraces were moved from one region to others, where they were given new names (Ntundu *et al*., 2004).

Table 2.28 Summary of Chi-square tests on the common names of landraces used as planting materials by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

	Local Government Areas													
	Bebeji		Gwarzo		Dambatta		Dawakin-Tofa		Rimin-Gado		Gabasawa		Gaya	
Class	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count
Local Names														
Hawayen Zaki	5	2.1	6	3.2	0	2.1	0	2.1	3	2.1	2	2.1	0	2.1
Mai-Yarfi	0	1.3	3	2	0	1.3	0	1.3	6	1.3	1	1.3	0	1.3
Fara	0	1.3	8	2	0	1.3	0	1.3	2	1.3	0	1.3	0	1.3
Baka	0	0.3	0	0.4	0	0.3	0	0.3	2	0.3	0	0.3	0	0.3
Ja	0	0.1	1	0.2	0	0.1	0	0.1	0	0.1	0	0.1	0	0.1
Idon Mikiya	2	0.4	1	0.6	0	0.4	0	0.4	0	0.4	0	0.4	0	0.4
Kundun Maiki	0	0.4	3	0.6	0	0.4	0	0.4	0	0.4	0	0.4	0	0.4
Hannun Marini	0	0.3	2	0.4	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
Ayaya	0	0.4	3	0.6	0	0.4	0	0.4	0	0.4	0	0.4	0	0.4
Kundun Zaki	2	0.3	0	0.4	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
Idon Muzuru	1	0.4	1	0.6	0	0.4	0	0.4	0	0.4	1	0.4	0	0.4
Balewa baka	1	0.4	0	0.6	0	0.4	0	0.4	2	0.4	0	0.4	0	0.4
Tamale Fulani	1	0.3	1	0.4	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
Kwaruru	4	0.5	0	0.8	0	0.5	0	0.5	0	0.5	0	0.5	0	0.5
Mai Koko	1	0.7	0	1	0	0.7	0	0.7	1	0.7	3	0.7	0	0.7
Dukusa	2	0.3	0	0.4	0	0.3	0	0.3	0	0.3	0	0.3	0	0.3
Idon Fara	1	0.4	0	0.6	0	0.4	0	0.4	2	0.4	0	0.4	0	0.4

Table 2.28 Continue

	Local Government Areas													
	Bebeji		Gwarzo		Dambatta		Dawakin-Tofa		Rimin-Gado		Gabasawa		Gaya	
Class	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count	Actual Count	Expected Count
Local Names														
Fareshi	0	0.1	0	0.2	0	0.1	1	0.1	0	0.1	0	0.1	0	0.1
'Yar cha-cha	0	0.7	0	1	1	0.7	4	0.7	0	0.7	0	0.7	0	0.7
Mai Bargo	0	0.8	0	1.2	0	0.8	6	0.8	0	0.8	0	0.8	0	0.8
"Yar Das	0	0.7	0	1	0	0.7	5	0.7	0	0.7	0	0.7	0	0.7
Gurjiya	0	2.9	0	4.4	7	2.9	1	2.9	0	2.9	12	2.9	2	2.9
Silva	0	1.2	0	1.8	1	1.2	0	1.2	0	1.2	0	1.2	8	1.2
Kyamuri	0	0.8	0	1.2	0	0.8	0	0.8	0	0.8	0	0.8	6	0.8
df	156													
X^2	515.428													
P-value	0.000													
Number of valid cases	20		30		20		20		20		20		20	

Legend: **df**=degrees of freedom

2.3.16 Farmers-preferred Bambara groundnut varieties

Farmers' preferences towards improved Bambara groundnut variety showed highly significant variation (P< 0.001) (Table 2.29). Overall, ten preferred traits were identified by the farmers. Most farmers preferred varieties with early maturity, high yield, pure and physically uniform coloured seeds. Farmers in Bebeji, Gwarzo and Gaya LGAs preferred early maturing varieties while high yielding varieties were required by farmers from Gwarzo, Rimin-Gado and Gabasawa LGAs. In Dambatta and Gwarzo most growers preferred varieties with pure seed. Early maturity, high yield and large seeded varieties were required by the farmers in Dawakin-Tofa LGA.

Requests by the Bambara groundnut farmers for improved varieties indicated the great need for the fulfillment of their agronomic needs. Berchie *et al*. (2010) reported that white and large seeds were preferred by Bambara groundnut farmers in Upper Regions of Ghana, while Abu and Buah (2011) reported fast cooking and early maturity were the most important attributes required by farmers in Ghana. Studies in Botswana, Namibia and Swaziland revealed that farmers' variety preferences include high yield, large seeds, earliness and spreading growth habit and fast cooking (http://www.wzw.tu-muenchen.de/pbpz/bambara/html/). These studies emphasized the need to identify a limited number of farmers' preferred traits that can be incorporated in a strategic breeding program.

Table 2.29 Summary of Chi-square tests on the preferred improved Bambara groundnut demanded by Bambara groundnut farmers from seven selected LGAs in Kano State, Nigeria

Local Government Areas	Class	Farmers' preferred traits						
		Earliness	High yield	Spreading	Large seed	Pure seed	Fodder	Good taste
Bebeji	Actual Count	12	5	0	2	1	0	0
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4
Gwarzo	Actual Count	12	10	0	2	4	1	1
	Expected Count	7.8	9.8	0.4	3.2	4.6	0.2	0.6
Dambatta	Actual Count	0	5	0	1	13	0	0
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4
Dawakin-Tofa	Actual Count	5	5	2	5	3	0	0
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4
Rimin-Gado	Actual Count	2	15	0	3	0	0	0
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4
Gabasawa	Actual Count	0	7	0	3	2	0	2
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4
Gaya	Actual Count	8	2	0	0	0	0	0
	Expected Count	5.2	6.5	0.3	2.1	3.1	0.1	0.4

Legend: **df**=degrees of freedom

Table 2.29 Continued

Local Government Areas	Class	Drought tolerance	Resistance to insects and diseases	Landrace	df	X^2	P-value	Number of valid cases
Bebeji	Actual Count	0	0	0				
	Expected Count	0.7	1.2	0.4				20
Gwarzo	Actual Count	0	0	0				
	Expected Count	1	1.8	0.6				30
Dambatta	Actual Count	0	1	0				
	Expected Count	0.7	1.2	0.4				20
Dawakin-Tofa	Actual Count	0	0	0	54	186.009	0.000	
	Expected Count	0.7	1.2	0.4				20
Rimin-Gado	Actual Count	0	0	0				
	Expected Count	0.7	1.2	0.4				20
Gabasawa	Actual Count	0	3	3				
	Expected Count	0.7	1.2	0.4				20
Gaya	Actual Count	5	5	0				
	Expected Count	0.7	1.2	0.4				20

Legend: **df**=degrees of freedom

2.3.17 Land area covered and harvestable yield of Bambara groundnut, cowpea, groundnut and soybean from the seven selected LGAs in Kano State, Nigeria

The farmland area planted to Bambara groundnut landraces showed highly significant variation ($P< 0.001$), while seed yields were not significantly different in the study area (Table 2.30). On average, more land area (in hectares) was allocated to Bambara groundnut in Dambatta LGA, followed by Bebeji (Table 2.31). These assessments were recorded based on famers' views. However, there was no difference in estimated yields among all the LGAs. Conversely, both land area and seed yield of cowpea differed significantly ($P< 0.05$), where the area covered and seed yields were higher from Gabasawa LGA followed by Gwarzo LGA. While both these crops are indigenous to Africa, and probably originated in West Africa (Begemann, 1988; Harlan, 1971; Hepper, 1963), variations between land area covered and seed yields may be associated with differences in the cowpea varieties used by the farmers, given that the cowpea breeding has received more research attention than Bambara groundnut. Also, the presence of the International Institute of Tropical Agriculture (IITA) sub-station in Kano may have assisted the farmers to access superior agronomic technologies for cowpea production, including varietal selection.

Both land area assigned to groundnut and soybean as well as grain yields were significantly different ($P< 0.001$) (Tables 2.30 and 2.31). Dambatta LGA had more land area apportioned to groundnut and higher seed yield, but there was more variability in grain yield than land area, which can be ascribed to the differences in availability and adoption of technology or environmental variability. On average, the Bambara groundnut farmers use relatively smaller portions of their land to groundnut production

in Rimin-Gado LGA. Akpalu *et al*. (2013) calculated that most Bambara groundnut farmers (40%) in their study area grow between 0.4 to 0.8 acres, while 6% grow 7 acres and above.

There was also highly ($P< 0.001$) significant difference among the selected LGAs on production area to soybean and harvest with Gwarzo the leading region, followed by Rimin-Gado. There was no report of production and harvest on soybean from Gabasawa and Dambatta LGAs, meaning that the crop is not important among the Bambara groundnut farmers in these two LGAs.

Table 2.30 Summary statistics of mean square and significant differences on estimated area grown with Bambara groundnut, cowpea and soybean with their harvested yield by the Bambara groundnut farmers from the seven selected LGAs in Kano State, Nigeria

		BBN		**BBNY**		**CWP**		**CWPY**	
Source of variation	df	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Between Groups	6	30.116	22.532**	500.164	1.541 NS	13.594	2.472*	262.745	2.025*
Within Groups	143	1.337		324.663		5.499		129.752	
Total	149								
		GNT		**GNTY**		**SBN**		**SBNY**	
Source of variation	df	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Between Groups	6	13.239	5.346**	1183.591	6.085**	12.306	8.732**	301.328	12.64**
Within Groups	143	2.477		194.512		1.409		23.838	
Total	149								

BBN=Bambara groundnut; **BBNY**=Bambara groundnut yield; **CWP**=Cowpea; **CWPY**=Cowpea yield; **GNT**=Groundnut; **GNTY**=Groundnut yield; **SBN**=Soybean; **SBNY**=Soybean yield*****Significant at P<0.05, ******Significant at P<0.001**, NS=**Not significant; **df**=degrees of freedom

Table 2.31 Mean area covered (Hectares) and yield (kg ha^{-1}) performances of Bambara groundnut, cowpea, groundnut and soybean from seven selected LGAs in Kano State, Nigeria

	Bambara groundnut		Cowpea		Groundnut		Soybean	
Local Government Areas	**Area**	**Yield**	**Area**	**Yield**	**Area**	**Yield**	**Area**	**Yield**
Rimin-Gado	0.38d*	802.5a	0.25b	187.5b	0.34c	370.0e	0.77a	692.5ab
Dawakin-Tofa	0.27d	730.0a	0.58b	960.0a	0.53bc	1135.0c	0.35bc	670.0cd
Gwarzo	0.58c	1766.67a	0.653a	481.67ab	0.81b	2076.7b	0.62ab	885.0a
Gabasawa	0.70c	1640.0a	0.80a	1195.0a	0.81b	1505.0cd	0.0 ND	0.0 ND
Bebeji	0.43cd	667.5a	0.31b	407.5b	0.83b	1782.5bc	0.47b	502.5bc
Gaya	1.02b	1405.0a	1.24a	790.0a	0.92b	660.0bc	0.14cd	125.0e
Dambatta	1.68a	1590.0a	0.62ab	920.0a	1.38a	2430.0a	0.0 ND	0.0 ND

*****Means in a column followed by the same letter(s) are not significantly different at the 5% probability level; **ND=**No data

2.3.18 Land area and harvestable yield of sorghum, millet, maize and rice from the seven selected LGAs in Kano State, Nigeria

There was highly (P< 0.001) significant difference in both production area assigned to sorghum and grain harvest among the Bambara groundnut farmers in the study area (Tables 2.32 and 2.33). More land area was allocated to sorghum in Gabasawa LGA from Zone III, followed by Dawakin-Tofa from Zone II. Conversely, grain yield was higher in Gabasawa from Zone II, followed by Bebeji from Zone I. These variations may possibly be associated to soil type since the LGAs were grouped in different Zones in which prevailing climatic conditions may vary. Highly (P< 0.001) significant difference was also observed among the Bambara groundnut farmers in millet production area and harvest (Tables 2.32 and 2.33). Statistically, Dawakin-Tofa, Gabasawa, Gaya and Dambatta apportioned bigger land area to millet production than Rimin-Gado, Gwarzo and Bebeji LGAs, but Dambatta and Gabasawa had led in harvestable grain yield. Both maize and rice land area of production and grain harvest showed highly (P< 0.001) significant differences among the Bambara groundnut farmers. Dawakin-Tofa was leading in maize production area followed by Rimin-Gado LGA. Gwarzo and Dawakin-Tofa had relatively higher grain yields, followed by Rimin-Gado. There was highly (P< 0.001) significant difference in rice production area and grain harvest in the study area. Bambara groundnut farmers in Rimi-Gado, Gabasawa and Gaya LGAs do not produce rice. Farmers from Dawakin-Tofa LGA assigned more land area to rice, but harvestable grain yield was higher in Bebeji LGA. Variations between production area and harvest may be attributed to the contrasting climatic conditions.

Table 2.32 Summary statistics of mean square and significant differences based on estimated area grown to sorghum, millet, maize and rice with their harvested yield by the Bambara groundnut farmers from the seven selected LGAs in Kano State, Nigeria

		SGM		**SGMY**		**MLT**		**MLTY**	
Source of variation	df	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Between Groups	6	49.689	12.2**	2114.959	8.846**	107.198	4.835**	3815.003	21.473**
Within Groups	143	4.073		239.092		22.173		177.666	
Total	149								
		MAZ		**MAZY**		**RCE**		**RCEY**	
Source of variation	df	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Between Groups	6	39.804	11.977**	3568.904	19.899**	4.055	4.08**	255.982	5.259**
Within Groups	143	3.323		179.349		0.994		48.674	
Total	149								

SGM=Sorghum; **SGMY**=Sorghum yield; **MLT**=Millet; **MLTY**=Millet yield; **MAZ**=Maize; **MAZY**=Maize yield; **RCE**=Rice; **RCEY**=Rice yield

Table 2.33 Means of area covered (ha) and yield (kg ha^{-1}) performances of sorghum, millet, maize and rice from seven selected LGAs in Kano State, Nigeria

	Sorghum		Millet		Maize		Rice	
Local Government Areas	**Area**	**Yield**	**Area**	**Yield**	**Area**	**Yield**	**Area**	**Yield**
Rimin-Gado	2.35c	1025.0d	0.35b	121.0e	2.825b	1825.0b	0.0 ND	0.0 ND
Dawakin-Tofa	3.695bc	2585.0b	5.18a	1735.0b	4.24a	2935.0a	0.755bc	345.0b
Gwarzo	2.365c	2263.3b	0.31b	220.0cf	2.6517cd	3410.0a	0.1167bc	767.0b
Gabasawa	6.525a	4270.0a	4.725a	2955.0a	0.3d	320.0c	0.0 ND	0.0 ND
Bebeji	2.625c	2880.0ab	0.525b	430.0d	1.6c	1325.0b	1.125a	975.0a
Gaya	4.8b	1625.0bc	3.7a	1030.0bc	0.4d	40.0c	0.0 ND	0.0 ND
Dambatta	3.275c	2710.0ab	4.2a	3435.0a	1.925cd	1630.0b	0.425c	445.0b

*****Means in a column followed by the same letter(s) are not significantly different at the 5% probability level; **ND=**No data

2.4 Conclusion

The present study is the first baseline survey conducted among the Bambara groundnut farmers in Kano State, Nigeria. During the survey, only farmers actively growing Bambara groundnut were chosen for the interview. It was observed that all the respondents interviewed were male. This was because due to the dominant cultural and religious mores of the region of Kano State, women are excluded from farming. However, women actively participate in the processing and cooking of farm produce within their matrimonial homes. This situation contrasts markedly with most regions in Africa, where most farmers are women. Mkandawire and Sibuga (2002), Ntundu *et al*. (2004), Massawe *et al*. (2005) and Clarke *et al*. (2010), reported that Bambara groundnut is mostly grown by women in other regions in Africa. Akpalu *et al*. (2013) carried out a survey in Upper East Region of Ghana and reported that 57% of the Bambara groundnut farmers were females, whereas 43% were males. Alhassan and Egbe (2013) observed 53% and 47%, being males and females, respectively, in a survey conducted in Benue and Kogi States, Nigeria, and Abu and Buah (2011) reported a mean of 97% females and 3% male farmers.

All the respondents were married, whereas Alhassan and Egbe (2013) found 95% and 5% being married and single, respectively, in Benue and Kogi regions. Gender differences among Bambara groundnut farmers indicated culture differences in the production areas. In Kano State Bambara groundnut was produced by one gender, male. This may be associated with both culture and religion. All the Bambara groundnut farmers interviewed currently grew the crop, and surplus pods and seeds were primarily sold on local markets. Large number of the respondents had Qur'anic education, which means that a large proportion of the farmers were not exposed to Western education.

The crop is important and popular in Kano State, Nigeria. However, important production constraints faced by the farmers include a lack of improved varieties, drought, low yields and limited market access and poor market prices. Collectively these problems may not be unconnected with lack of sufficient genetic enhancement of the crop that limits the production and release of desirable planting materials to the growers. The farmers sell their surplus pods and seeds in the open or local markets.

Choice of landraces among the farmers differed; however, most farmers preferred oval and large pure seeds with a cream-yellow seed coat colour and early maturity. Abu and Buah (2011) reported that farmers in Ghana selected Bambara groundnut landraces based on features including seed coat colour, seed yield, seed size and size shape, maturity and growth habit, and pest resistance. The aforementioned chosen characters were not based on Bambara groundnut descriptors (IPGRI/IITA/BAMNET, 2000), but on farmers' opinions. Farmers preferred improved variety based on the characteristics they choose have breeding implication if new varieties are to be bred to meet the needs of the end users, the farmers.

However, to meet these demands, Bambara groundnut landraces need to be sorted into seed morpho-types by seed and pod colours, shapes, sizes, etc. so as to have homogenous materials as a starting point for the systematic breeding of this crop.

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CHAPTER THREE

Phenotypic characterization of diverse Bambara groundnut germplasm collections through seed morphology

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is an important grain legume native to Africa. Unlike other legumes, the crop has been largely neglected by science. In Africa, farmers currently grow unimproved and heterogeneous landraces in seed mixtures that hold distinctive and divergent genetic attributes. The systematic selection of Bambara groundnut landraces into defined homogenous groups of seed morpho-types for effective breeding would boost crop productivity and quality, and improve food security. Systematic pre-breeding of Bambara groundnut is a starting point to enhance the productivity of the crop. The objective of this study was to characterize a wide range of germplasm of Bambara groundnut collections using seed morphology to classify and identify unique germplasm. Bambara groundnut seed collections (58 seed lots) from seven diverse geographic origins were phenotyped using visual technique to describe seed morphological features including: seed coat colour and pattern, seed eye colour and pattern and hilum colour and pattern. The study generated baseline seed morphology diversity information, and 353 different seed morpho-types of the crop were distinguished for field production of true to type lines and further genetic improvement.

Keywords: Bambara groundnut, landrace, pre-breeding, seed morphology

3.1 Introduction

Low agricultural productivity, population pressure and climate change are driving food insecurity, malnutrition and poverty in continental Africa (Eitzinger *et al*., 2010). However the region is endowed with unique crops that can grow in harsh environments and provide unique nutritional value. Some of the potentially useful crop species, however, are underutilized and have not been scientifically evaluated and bred as food crops (Padulosi *et al.*, 2002). Among these crops is the Bambara groundnut (*Vigna subterranea* Verdc., 2n=2x=22), which is well-adapted to a wide range of growing conditions in Africa, from marginal, drought-prone environments to those of high potential.

Bambara groundnut is rich in carbohydrates (63%), protein (20%) and oil (18%) (Rowland, 1993). Its protein contains essential and non-essential amino acids at 32.7% and 66.1%, respectively. These include lysine and methionine at 6.82g/16gN and 1.85g/16gN, respectively (Fetuga *et al*., 1975). Bambara groundnut is traditionally eaten as a boiled bean, or added to stews, or it can be made into a sweetened pudding. The flour

has strong water and oil binding qualities, and it is therefore widely used to make indigenous bread, or to create a milk, similar to soya milk (Okpuzor *et al.*, 2010). The young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanut, and can be made into pudding (or steamed-paste) which is called Moi-Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzor *et al.*, 2010). In Zambia, Bambara groundnut is used for bread making (Brough *et al.*, 1993), and milk (Poulter and Caygill, 2006, cited by (Okpuzor *et al.*, 2010). Dried seeds can be roasted and eaten as confectionery. Due to its nutritional importance Bambara groundnut is an ideal crop for rural communities where high protein animal products are not readily available or affordable for consumption (Linnemann and Azam Ali, 1993).

Bambara groundnut originated in West Africa and has considerable genetic diversity. The crop is widely distributed and grown throughout Africa where small scale farmers currently grow unimproved and heterogeneous landraces. The genetic potential of the crop could be enhanced through targeted breeding to boost productivity, product quality and improve food security. Systematic pre-breeding of Bambara groundnut is a starting point to enhance the productivity of the crop. Given that this has not taken place previously, and its wide genetic diversity, substantial gains should be made relatively quickly. Thus, future research should focus on the pre-breeding and breeding of this crop to its genetic potential, followed by the dissemination of seed of improved varieties to farmers.

Bambara groundnut is usually intercropped with cereals, and root and tubers crops. As a sole crop the yield of the crop varies from 650-850 kg ha^{-1}, but yield potential of >3000 kg ha^{-1} was reported (Collinson *et al.*, 2000). Bambara groundnut can outyield most other legumes under severe growing conditions.

Bambara groundnut was probably domesticated from its wild relative, *Vigna subterranea* var. *spontanea* as a result of gradual changes via natural and artificial selection (Doku and Karikari, 1971). The production and consumption of Bambara groundnut is largely confined to the semi-arid regions of Africa where rainfall is unreliable and low. The crop is also cultivated in America, Asia and Australia (Suwanprasert *et al.*, 2006).

In spite of the various economic advantages of Bambara groundnut, it remains a neglected and under-utilized crop species in sub-Saharan Africa (Massawe *et al.*, 2005). This is associated with the lack of research attention by scientists at national and international level to improve the crop, unlike other legume crops such as groundnut and cowpea (Massawe *et al.*, 2005) (Massawe *et al.*, 2005), there is scanty information on the genetic evaluation of the crop, using the diversity of seed morphology as a basis for selection and for systematic crop improvement by classic plant breeding.. In the absence of improved varieties, farmers grow landraces which are heterogeneous seed mixtures, resulting in variable yields between years and localities (Abu and Buah, 2011). Neglected and underutilized crops, such as Bambara

groundnut, could play a prominent role in sustaining the livelihood of poor rural African populations by increasing food availability, including protein uptake (Padulosi *et al.*, 2002).

Strategic collection, characterization and preservation of genetic resources are major components in plant breeding programs, especially with new and under-utilized crops (Traka-Mavrona *et al.*, 2000; Olukolu *et al.*, 2012). This will help for targeted breeding involving various characteristics and for germplasm conservation.Careful selection and classification of the Bambara groundnut germplasm is important using seed morphology and important agro-morphological attributes. Seeds of Bambara groundnut landraces possess identifiable morphological features, such as seed testa colour, seed shape, seed eye, and hilum colour and pattern. Farmers' selection of Bambara groundnut seed in Ghana have centered on seed morphological features including seed coat colour, yield, size, shape, and plant maturity (Abu and Buah, 2011). The morphological features of Bambara groundnut can be utilized for its genetic improvement upon classification into homogenous seed material. The objective of this study was to characterize a wide range of germplasm of Bambara groundnut collections from seven geographical zones across Africa using seed morphology to classify, and identify unique germplasm. Results of the study may be valuable to generate baseline seed morphology diversity information in the strategic breeding of the crop.

3.2 Materials and Method

3.2.1 Bambara groundnut germplasm collection

Seeds of Bambara groundnut germplasm were obtained from various national research and development programs including Zimbabwe, Zambia, South Africa, the International Institute of Tropical Agriculture (IITA) and farmers' collections from Kano, Nigeria (Table 3.1). A total of 25 landrace collections were received from Zambia (the largest collection), followed by those from IITA and Zimbabwe with 14 and 12, respectively. Other collections were secured from a farmer in Pietermaritzburg and from Capstone Seed Company (CAPS) in Howick, South Africa. The total seed collection was 58, which represented seven geographical collection centers. Landraces sourced from IITA and their origins were presented in Table 3.2. The collections were received as single seed lots bearing landrace names. Diversity score was used to calculate the extent of deviation of new morpho-types from the initial collection as follows:

Diversity score = Number of new morpho-types / Number of initial collection

Table 3.1 Source, number of initial collections, new morpho-types and diversity score of the Bambara groundnut landraces used in the study

Source	Initial Collection	New morpho-types	Diversity Score	Rank
Zimbabwe (ZIM)	12	46	3.8	6
Zambia (ZAM)	25	135	5.4	5
Agricultural Research Council (ARC)	3	17	5.7	4
Pietermaritzburg, farmer's field (PMB)	1	38	38.0	2
Capstone (CAPS)	1	77	77.0	1
IITA*	14	18	1.3	7
Kano, Nigeria farmers' fields (KNG)	2	22	11.0	3
Total	**58**	**353**		

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa; * Originated from 9 countries and regrouped in Table 2

Table 3.2 Landrace collections sourced from IITA and their country of origin

Serial Number	ID number	Source
1	TVSu-20	Nigeria
2	TVSu-275	Nigeria
3	TVSu-570	Nigeria
4	TVSu-571	Nigeria
5	TVSu-390	Sudan
6	TVSu-391	Sudan
7	TVSu-1466	Ghana
8	TVSu-118	Côte d'Ivoire
9	TVSu-1900-1	Zambia
10	TVSu-1900-2	Zambia
11	TVSu-1900-3	Zambia
12	TVSu-85	Burkina Faso
13	TVSu-792-1	Kenya
14	TVSu-792-2	Kenya
15	TVSu-792-3	Kenya
16	TVSu-793	Kenya
17	TVSu-290	Benin
18	TVSu-1778	Malawi

3.2.2 Seed phenotyping and identification

The 58 seed lots were sorted separately and in a similar way (Fig. 3.1 ‘1 to 4’) starting with same seed colour groups, and seed eye colour and pattern as indicated in Fig. 3.1 ‘5 to 34’ . This was followed by eye pattern description imposed on the classification by seed coat colour morpho-types (Fig. 3.2 ’A to Y’). Similar procedure was employed to classify Bambara groundnut landrace accessions at IITA based on seed features including seed eye and hilum colour and pattern (Mkandawire, 2007).

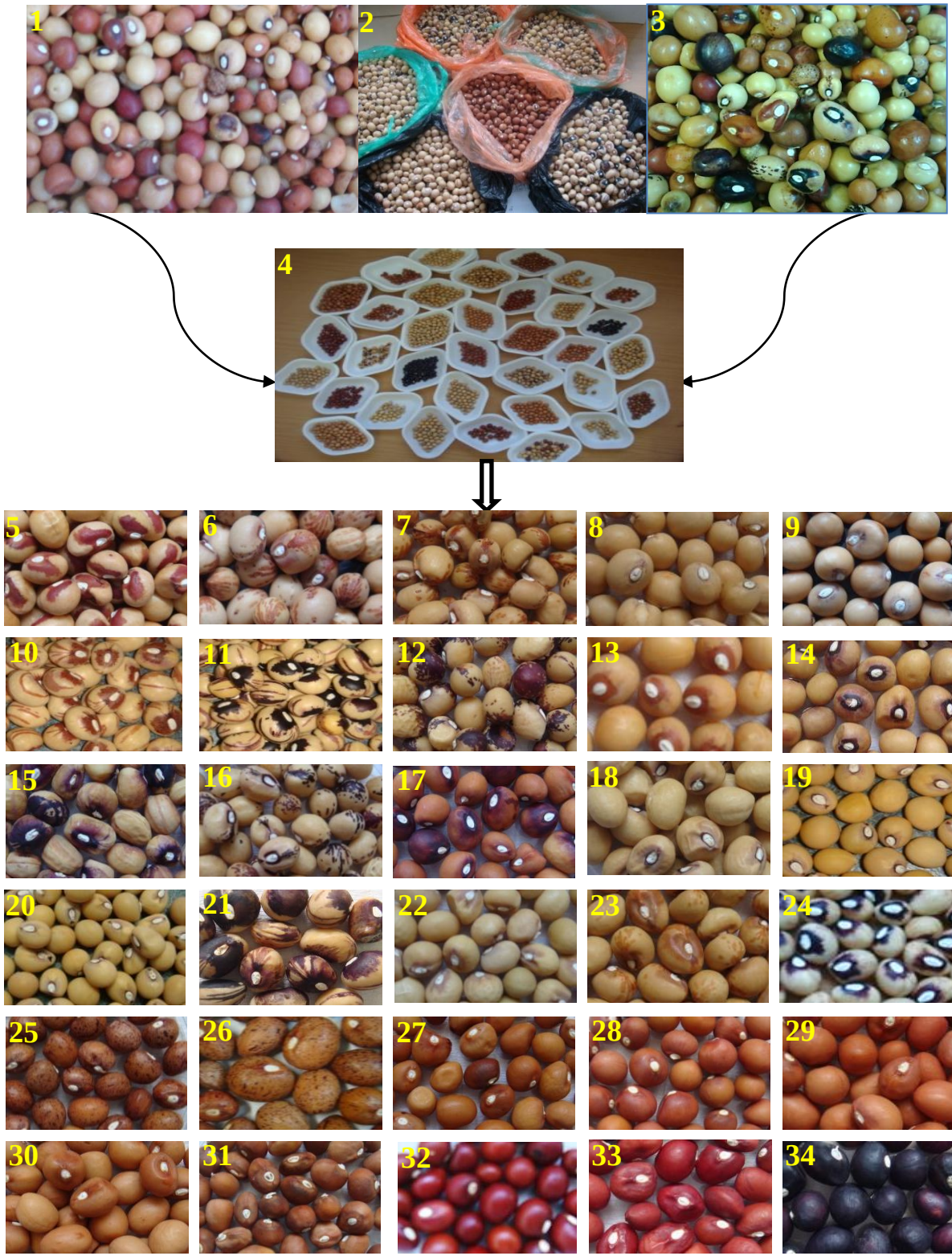

Fig. 3.1 Stages of Bambara groundnut landrace classification into homogenous seed morpho-types using seed coat colour: 1, 2 and 3 are general seed features of Bambara groundnut landraces; 4 shows sorted seed colour groups; and 5 to 16 and 18 to 24 shows variations among cream seed coat colour groups; 17 brown coat coloured landrace with purple eye; 25 and 26 shows speckle brown seed coat; 27 to 31 shows brown seed coat colour groups; 32 and 33 red seed coat landraces; and 34 shows black seed coat landrace

Fig. 3.2 Bambara groundnut landraces assorted by seed eye colour and hilum pattern: A to J, show some variations of eye pattern among cream-coloured Bambara groundnut landraces; K shows an exceptional 'curved-in' brownish hilum, without an eye; D and E show variations between two butterfly-eyed landraces, black and red, respectively; H, I and J show cream coloured landraces with striped purple, light brown grey (broadened) and striped black eye patterns, respectively; M shows a typical black landrace, with no hilum; U shows a light-cream coloured landrace with 'Chalk-white' hilum; X shows a brown speckled landrace without eye; and L, M, O, P, Q, R, S, W and X possess the most frequent hilum colour (white) and without eye colour among the classified landraces (Table 3.6)

Furthermore, similar procedure applied seed lost assorted for landraces collected from Kano, Nigeria. Their identity was assigned as KN 211-1, -2, -3 to the last seed lot; and for 2011 collections and KN 212 for 2012 collections. After seed assortment data were summarized in Table 3.3.

Table 3.3 Summary of seed morpho-types of Bambara groundnut landraces

Name of landrace	ID number	Source	Seed coat colour	Seed eye pattern	Seed hilum colour
ZM 101-1	M 01-1	Zimbabwe	Cream	Brown-broad	White
ZM 101-2	M 01-2	Zimbabwe	Cream	Black-broad	White
ZM 102-1	M 02-1	Zimbabwe	Cream	Purple/black thin	White
ZM 102-2	M 02-2	Zimbabwe	Cream	Black-thin	White
ZM 105-1	M 05-1	Zimbabwe	Black	Plain	White
ZM 105-2	M 05-2	Zimbabwe	black-speckle	Plain	White
SB 7-2	B 71-2	ARC-RSA	Red	Plain	White
SB 7-1-3	B 71-3	ARC-RSA	Dark-red	Plain	White
KUBU 06	KB 06	ARC-RSA	Cream	Light brown-thin	White
KUBU 07	KB 07	ARC-RSA	Cream	Light brown-thin	White
SB 19-3-2	19-3-2	ARC-RSA	Black	Plain	White
SB 19-3-3	19-3-3	ARC-RSA	Dark-grey	Plain	White
ZM 4673-1	73-1	Zambia	Cream	Light-grey	White/Black
ZM 4673-2	73-2	Zambia	Brown	Plain	White
ZM 6608-1	608-1	Zambia	Tan	Light brown-thin	White
ZM 4675-4	75-4	Zambia	Cream	Greyish	White
ZM 4675-5	75-5	Zambia	Cream	Black-butterfly	White
ZM 2045-1	45-1	Zambia	Cream	Black-broad	White/curved-in
ZM 3643-1	43-1	Zambia	Whitish-cream	Plain	Chalk-white
ZM 3643-2	43-2	Zambia	Whitish-cream	Light brown	Chalk-white
PMB 011-1	011-1	PMB	Cream	Black-butterfly	White
PMB 011-2	011-2	PMB	Cream	Grey-broad	White
PMB 011-6	011-6	PMB	Cream	Red-butterfly	White

Note: Bold faced fonts denote '*the original landrace names and IDs, and names and IDs that follow are the sorted morpho-types within the original landraces'*

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

Table 3.3 Continued

Name of landrace	ID number	Source	Seed coat colour	Seed eye pattern	Seed hilum colour
TVSu-1900-1	TV-19-1	Zambia	Cream	Light-brown broad	White
TVSu-1900-2	TV-19-2	Zambia	Cream	Black-broad	White
TVSu-1900-3	TV-19-3	Zambia	Cream	Black-broad	White
TVSu-792-1	TV-79-1	Kenya	Brown	Plain	White
TVSu-792-2	TV-79-2	Kenya	Brown	Plain	White
TVSu-792-3	TV-79-3	Kenya	Brown	Plain	White
KN 211-2	N 211-2	Kano Nigeria	Cream	Light-grey	White
KN 211-3	N 211-3	Kano Nigeria	Cream-brown stripe	Dark-brown	White
KN212-14	N 212-14	Kano Nigeria	Black/white stripe	Plain	White
KN212-15	N 212-15	Kano Nigeria	Purple/black stripe	Grey	White
PSC 211-66	211-66	CAPS	Light-brown	Plain	White
PSC 211-66-1	211-66-1	CAPS	Light-brown	Plain	White/curved-in
PSC 211-66-2	211-66-2	CAPS	Brown speckle	Plain	White
PSC 211-86-1	211-86-1	CAPS	Cream	Light brown	White/curved-in
PSC 211-86-2	211-86-2	CAPS	Cream brown-stripe	Light brown	White

Note: Bold faced fonts denote '*the original landrace names and IDs, and names and IDs that follow are the sorted morpho-types within the original landraces'*

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

However, landrace collections from Zambia, ARC and IITA which had an initial identification or 'landrace name' were identified as such. Where there were variants or 'morpho-types' from a seed lot, initials of '-1, -2, -3 and so on were assigned to identify the respective morpho-types. Landraces collections from a farmer in Pietermaritzburg acquired in 2011 were identified as PMB 011-1, PMB 011-2, and so on, to distinguish variants or morpho-types.

3.3 Results

3.3.1 Variations in seed coat colour and pattern

The Bambara groundnut landraces varied widely in the seed coat colour and pattern. Thirty descriptors were used to differentiate all the landraces from the seven geographical locations (Table 4). Seed coat colours identified include cream, black, red, brown and tan of various brands. The results show that there are more cream seeds based coloured landraces among the Zambian landraces (56) morpho-types, followed by collections from a farmer field in Pietermaritzburg area having 28 seed coat coloured landraces. Farmers' field collections from Kano, Nigeria and those of ARC had the least variation, having seven landraces with cream seed coat coloured landraces. A total of 147 cream coloured landraces were classified as most common seed colour followed by brown based seed coat colours with 65 landraces. Several rare cases were also observed (Table 3.5).

Table 3.5 Classification of Bambara groundnut landraces based on seed coat colour and pattern

S/No.	Seed coat colour and pattern	Source of collection							TOTAL
		ZIM	ZAM	ARC	PMB	KNG	IITA	CAPS	
1	Cream	15	56	7	28	7	10	24	147
2	Cream red stripe	2			2			3	7
3	Cream black stripe				1		1		2
4	Cream purplish	1							1
5	Cream-brown/purplish stripe					1			1
6	Cream light-brown stripe							1	1
7	Cream brown-stripe					8			8
8	Cream light-grey broad						1		1
9	Cream light-grey spots							1	1
10	Cream dark brown patches		1						1
11	Whitish cream		7				1		8
12	Black	7	7	2	5		4	2	27
13	Black white-speckle	1							1
14	Grey brown			1					1
15	Dark grey			1					1
16	Purple brown					1			1
17	Red	7	9	3				1	20
18	Light red		1						1
19	Dark red							2	2
20	Brown	8	35	2	1	4	4	11	65
21	Dark brown	1		1			1	10	13
22	Light brown		1			1		5	7
23	Brownish cream		2						2
24	Brown black-stripe						1		1
25	Brown black spots							3	3
26	Dark brown speckle				1		2		3
27	Dark brown black spots							5	5
28	Brown dark-speckle	1	1					1	3
29	Tan	3	11				1	4	19
30	Variegated cream/black		1					1	2
	TOTAL	**46**	**131**	**17**	**38**	**22**	**25**	**74**	**353**

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

3.3.2 Variations in seed eye colour and pattern

Thirty descriptors were used to classify the landraces for seed eye colour and pattern. The result showed that a total of 180 of the landraces had plain eyes (Table 3.6). Landraces with a plain eye pattern were only composed of the uniform seed coat colour and hilum (Fig. 3.2 'O, P, Q, R, S, U and W'). There were more of the plain eyed landraces from the Zambian collection with 73 morpho-types, followed by landraces from CAPS with 40 morpho-types (Table 3.3).

Table 3.6 Classification of Bambara groundnut landraces based on seed eye colour and pattern

S/No.	Seed eye colour and pattern	Source of collection							TOTAL
		ZIM	ZAM	ARC	PMB	KNG	IITA	CAPS	
1	Black broad	4	10		3	1	2	3	23
2	Black thin	7	5		3			1	16
3	Black-light grey thin							1	1
4	Black broad stripe					1			1
5	Black butterfly	1	2		1				4
6	Red butterfly				3			2	5
7	Brown stripe thin				1				1
8	Brown broad	1	13		6		1	2	23
9	Brown thin		8		1		2	1	12
10	Brownish grey thin		1						1
11	Dark brown broad					2		2	4
12	Dark brown thin		1			2		2	5
13	Dark brown grey thin							1	1
14	Grey broad		1		5		1	5	12
15	Grey thin		4						4
16	Light brown grey broad		1						1
17	Light brown broad		1		2		2	1	6
18	Light brown thin	3	8	7		5		2	25
19	Light grey broad	1			1		1	1	4
20	Light grey thin				1	4		5	10
21	Light dark thin							4	4
22	Red broad		2						2
23	Red thin		1		1				2
24	Red grey broad				1				1
25	Red grey thin				1				1
26	Red light grey thin								
27	Light red broad							1	1
28	Plain	27	73	10	8	6	16	40	180
29	Purple thin	1				1			2
30	Purple broad	1							1
	TOTAL	**46**	**131**	**17**	**38**	**22**	**25**	**74**	**353**

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

3.3.3 Variations in seed hilum colour and pattern

Bambara groundnut landraces from the seven geographical locations were characterized for seed hilum colour and pattern using six descriptors (Table 3.6). The result indicated that 92.4 % of the landraces had a white eye (Table 3.7) and (Figs. 3.2 A to K), except for E and L (Fig. 3.2) representing 0.6 % of the total which had brownish hilum. This was followed by chalk-white hilum which consists of 11 landraces representing 3.1 % of the total (Table 3.6). Landraces with chalk-white hilum pattern were composed of this basic seed coat colour, no eye, while the hilum was exceptionally white or chalk-white (Fig. 3.2 M). Another interesting hilum feature was the 'curved-in' hilum pattern. Ten landraces with this feature were identified. Curved-in refers to a hilum that was sunken or depressed (Fig. 3.2 E)..

Table 3.7 Classification of Bambara groundnut landraces based on seed hilum colour and pattern

Seed hilum colour and pattern	Source of collection								
	ZIM	ZAM	ARC	PMB	KNG	IITA	CAPS	TOTAL	% of Total
Brown		2						2	0.6
Chalk-white		8			1	2		11	3.1
White	44	121	17	37	21	23	63	326	92.4
White/black dot							1	1	0.3
White/red dot							1	1	0.3
White curved-in	2			1			7	10	2.8
TOTAL	**46**	**131**	**17**	**38**	**22**	**25**	**74**	**353**	

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

Number of landrace collection for the seven geographical zones including the IITA accessions vary greatly. Initial collection showed that, more accessions were received from Zambia with 25 seed lots (Table 3.1). Accessions acquired from IITA and Zimbabwe had 14 and 12 seed lots each, respectively. Farmer landrace collection from PMB and that of CAPS were the least with 1 seed lot each. Fifty eight seed lots of Bambara groundnut landraces (Table 3.1) were acquired in total. After sorting and classification, available number of morpho-types was 353 with Zambia having the highest number of morpho-types at 134. Unlike the initial collections record, landrace collections from CAPS and Zimbabwe followed with 77 and 46 seed morpho-types each, respectively. The least variation was found in the landrace collection from IITA which had 14 and rose to 18 (Table 3.1).

3.4 Discussion

This is one of the few reports that presents the classification of the diversity of Bambara groundnut landraces based on seed morphology. Similar report was earlier presents by Mkandawire (2007). Seeds of the Bambara groundnut collections in this study displayed numerous variations with respect to the morphological features used for classification. Out of the 12 and 25 seed lots from Zimbabwe and Zambia, 46 and 134 morpho-types were observed, representing diversity scores of 3.83 and 5.40, respectively (Table 3.1). Initial collections from the Capstone Seed Company with only one seed lot ranked 1st and had the high number of seed morpho-types, representing 77.0. This means that the CAPS buy in and sales Bambara groundnut seed which is composed of heterogeneous mixtures of diverse seed morpho-types.

The Farmer's collections from PMB, South Africa and that from Kano, Nigeria ranked 2nd and 3rd in diversity, with diversity scores of 38.0 and 11.0, respectively. This revealed that Bambara groundnut farmers from these two agro-ecologies produce this crop from seed mixtures. The least variation of morpho-types was recorded for accessions from IITA which had diversity score of 1.7. Although IITA has an autonomous mandate for germplasm conservation, yet little attention has been accorded to the extensive characterization of Bambara groundnut germplasm. The diversity of the crop remains largely unexploited especially at institutional levels (Massawe *et al.*, 2005). However, there appeared to be some level of seed sorting with the IITA materials. From Table 3.2, all the IITA seeds sourced from nine countries were uniform and homogenous except for collections from Kenya and Zambia, which consisted of three distinct morpho-types each. From a total of 58 collections representing seven different sources, 353 morpho-types were observed displaying an average diversity score of 6.09 (Table 3.1). The seed morphological diversity of the Bambara groundnut landraces used in this study varied greatly. Variation in seed features including seed coat colour and eye pattern, and hilum colour and pattern have been previously reported (Massawe *et al.*, 2005; Abu and Buah, 2011). These authors confirmed that Bambara groundnut landraces possess distinguishable morphological identities that can be exploited through breeding. In this study, 30 descriptors for seed coat colour were used where cream, black, red, brown and tan base seed coat colours of various assortments were observed. Landraces bearing cream colour dominated. These were observed among the Zambian landraces, with 56 out of the 353 classified morpho-types (Table 3.5). Out of the 353 morpho-types, 147 had cream coloured seed coat. Brown seed coat colour followed with 65 morpho-types, while other colours had fewer representations. Also, 30 descriptors to classify the Bambara groundnut landraces were employed for variations in seed eye colour and pattern (Table 3.6). The result indicated that out of the 353 morpho-types, 180 landraces had a plain eye, followed by light-brown seed eye colour and pattern. Black and brown eyes had 23 each. The

variations in seed coat colour and eye colour and patterns displayed by the landraces are useful to differentiate between the germplasm in a program of genetic improvement of Bambara groundnut (Padulosi *et al.*, 2002). Despite domination by the aforementioned morphological features, the rare variants will bear equally useful genetic information that can be exploited through breeding.

Furthermore, the Bambara groundnut landraces presented diverse hilum colours and patterns; in which 326 of the landraces had white hilum, while 11 were chalk-white in colour (Table 3.7). Pattern wise, 10 landraces were observed to possess a curved-in pattern of hilum.

Seeds of Bambara groundnut landraces possess identifiable morphological features, such as seed testa colour, shape, eye, and hilum colour and pattern (Abu and Buah, 2011). Farmers' selection of Bambara groundnut seed in Ghana (Abu and Buah, 2011) have centered on seed morphological features including seed coat colour, yield, size, shape, and plant maturity. Reportedly, the crop shows enormous genetic variation in Africa and a large number of Bambara groundnut landraces are still being selected and preserved by small-scale farmers (Massawe *et al.*, 2005).

3.5 Conclusion

The genetic morphology of a collection of Bambara groundnut seed was determined in this study. The indices used for the morphological classification, included seed coat colour and pattern, seed eye colour and pattern, and hilum colour and pattern. The landraces possessed numerous variants of morpho-types with respect to the procedure used for their classification. The classification procedure was used as a starting point of pre-breeding, which is a basic requirement for the enhancement of the Bambara groundnut for yield and yield stability, seed quality and resistance. The study generated a baseline of seed morphology diversity information where 353 different seed morpho-types of the crop were distinguished for field selection of true to type lines for further genetic improvement.

3.6 References

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CHAPTER FOUR

Agro-morphological variation within and between Bambara groundnut landraces

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is an indigenous legume crop in Africa. It has comparable value to other legumes for food and nutritional security in the continent. However, small-scale farmers continue cultivating unimproved landrace varieties over the production areas in sub-Saharan Africa. Bambara groundnut landraces exist as heterogeneous mixtures of seeds, which typically contain a few to several seed morpho-types that may embrace wide genetic diversity. In this study, the agro-morphological variations of 262 Bambara groundnut landraces were evaluated to determine the genetic variability present within- and between-landraces for agronomic traits (using 49 landraces) and pod and seed morphology involving 213 landraces. Most of the landraces displayed pointed and round and yellowish pod colour, with grooved and oval seed shapes. Out of the 158 landraces accessed for leaf morphology, 49.4% had round leaves, while 21.5% had elliptical leaves, with 55.7% landraces that were heterogeneous and possessing more than one leaf shapes. Significant variations were detected for pod and seed traits. Leaf morphology could be a useful marker for strategic breeding and genetic conservation of Bambara groundnut.

Keywords: Agronomic traits, Bambara groundnut, breeding, landrace, morphology

4.1 Introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc.; **Syn:** *Voandzeia subterranea* [L.] Thouars.) is an African grain legume widely grown in arid and semi-arid regions (Goli *et al*., 1997). West Africa is believed to be the centre of diversity of the crop (Hepper, 1963). Bambara groundnut is also grown in Sri-Lanka, Malaysia, Philippines, India and Brazil (Rassel, 1960; Goli *et al*., 1997). The crop is mainly grown by subsistence farmers under traditional agricultural systems, mostly for home consumption (Abu and Buah, 2011). Bambara groundnut is an under-utilized legume crop and grows as landrace varieties with unpredictable and low yields.

Bambara groundnut has multiple advantages comparable with that of other legumes such as cowpea, dry bean, and groundnut. The seed of Bambara groundnut is rich in protein and this complements the cereal based diets of most rural communities in Africa (Ntundu *et al*., 2004; Olukolu *et al*., 2012). Chemical analyses of the seed revealed that about 32.7% of essential amino acids comprise of lysine, histidine, arginine, leucine and isoleucine, while 66.1-70.8% were non-essential amino acids including methionine,

glycine, cysteine, tyrosine and proline (Minka and Bruneteau, 2000; Amarteifio *et al*., 2010). In its fresh form, the seed is consumed as vegetable, while dry seed can be processed to flour to prepare various kinds of foods including Moi-Moi (a form of steamed-paste) in Nigeria (Okpuzor *et al*., 2009). Dry seeds are also used as animal feed (Ntundu *et al*., 2006).

The crop is tolerant to drought, adapts to severe environment and has the ability to produce some yield where other legumes may not grow well. It also suffers attack from few pests and diseases (Azam-Ali *et al*., 2001; Sesay *et al*., 2008). Bambara groundnut has the ability to fix atmospheric nitrogen into the soil through symbiotic activity with *Rhizobium* sp., which is highly beneficial when grown in rotation with cereal crops (Karikari *et al*., 1999). Although yield of Bambara groundnut is unpredictable (Massawe *et al*., 2002), the crop has the potential to produce up to 3,000 kg ha^{-1} (Collinson *et al*., 2000). Seed yield between 700 to 1000 kg ha^{-1} has been reported in Ghana on famers' field (Abu and Buah, 2011), in which farmers were observed to plant mixed seeds (landraces) as an approach to at least make some harvest in times of weather uncertainty (Brink *et al*., 2000). Despite its values Bambara groundnut has not receive sufficient research attention. As a result there is no coordinated effort for agronomic improvement of the crop through breeding (Ntundu *et al*., 2004). More research resources have been devoted to cereal crops such as maize, millet and sorghum, and to other legumes, especially groundnut, dry bean and cowpea (Drabo *et al*., 1995). The lack of genetic variability and the absence of suitable ideotypes that are adapted to specific cropping systems are additional constraints limiting seed yields (Sprent *et al*., 2010). Therefore, genetic enhancement and breeding is needed through the utilization of available germplasm.

Previous reports indicated the presence of within and between landrace variability (Massawe *et al*., 2002; Massawe *et al*., 2003) that can be exploited in breeding. Well-characterised germplasm is essential for strategic conservation and genetic enhancement through pre-breeding and breeding techniques. Bambara groundnut has varied names such as Jugo beans or *Indlubu* (South Africa), *Gurjiya* or *Kwaruru* in Hausa (Northern Nigeria) (http://en.wikipedia.org/wiki/*Vigna_subterranea*/) and in Swahili, it is known as *Njugumawe* (Hillocks *et al*., 2012). Bambara groundnut landraces are usually named in relation to the site of their collection, such as the markets where they were purchased, or their seed coat colours, neither of which reflect their origin (Massawe *et al*., 2002). Thus one landrace may be grown in several growing regions with many names. Thus far no improved varieties have been released following a well-designed breeding of the crop. Farmers typically practice a crude form of mass selection and retain their own seed from season to season, often with mixed seed morpho-types. Some distinguishable features of the landrace varieties grown by farmers include seed morphology, which may be round or oval in shape. These traits can be utilized to initiate selection and phenotypic evaluation through field characterization that would further be used for breeding and systematic conservation. Selection of desirable genotypes

increases their use in breeding program to improve selection response on agro-morphological traits. The integration of under-utilized species such as Bambara groundnut landraces in the agro-biodiversity research and conservation would assist in mitigating climate changes and ensuing global food security (Jaenicke, 2011). For improved productivity of a crop species, genotypes possessing uniform growth and reproduction are selected, bred and released for large scale production (Rauf *et al*., 2010). Characterization of Bambara groundnut landraces as source of desirable genes is a primary step towards the conservation of biodiversity and for effective breeding (Ghalmi *et al*., 2010).

In this study the agro-morphological variation of 262 Bambara groundnut landraces were evaluated to determine the genetic variability present within- and between-landraces, for agronomic traits (using 49 landraces), and pod and seed morphology (using 213 landraces). The seeds were a selection from a study of the diversity of Bambara groundnut using seed morphological features of the Bambara groundnut landraces, presented in the previous chapter (Chapter III).

4.2 Materials and methods

4.2.1 Study site

The study was carried out in the field at the Ukulinga Research and Training Farm of the University of KwaZulu-Natal (UKZN), and in the controlled environment facility of UKZN Pietermaritzburg campus, South Africa. The experiments were conducted from October, 2011 to May, 2012. The field site is situated on a Latitude 30^{o} 24'S, Longitude 29^{o} 24'E, and 800 m above sea level (Information was provided by the University weather station).

4.2.2 Plant material, experimental design, field management, and data collection

Forty nine genotypes of the Bambara groundnut landraces were used for the field experiment. The landraces were evaluated using a partially balanced lattice design with two replications (Table 4.1). The genotypes were randomized within seven incomplete blocks over the two replications. The experimental plot comprised of a single row measuring 2.2 m long, with inter- and intra-row spacing of 0.4m x 1.0m, respectively. This spacing was referred to be sufficient to allow the crop to express its potential in the field. Each row represents a plot.

Further a set of 105 landraces were grown in the field in a non-replicated trial which were used for the assessment of leaf morphology. Another set of 55 landraces were included and grown in plastic pots in the greenhouse. In summary, 213 entries were included for the determination of qualitative traits among pods and seeds. In the field, sowing was done on flat bed, with one seed sown to each stand. Missing stands were replaced within two weeks after sowing. All relevant agronomic practices were carried out to

maintain a healthy crop. The entire selected landraces represent landrace collections from six geographical zones of sub-Saharan Africa (Tables 4.1 and 4.5).

Data on quantitative agronomic and seed traits were collected. Data on the quantitative traits from the replicated trial were generated using ten tagged plants in each row within the seven incomplete blocks over the two replicates as well as from the non-replicated trial. The quantitative field data included number of days to 1st seedling emergence (SEM) and number of days from planting to 50% seedling emergence (FPEM). These were taken as number of days from sowing to seedling emergence. Other measurements were taken using a measuring ruler expressed in centimeter (cm), including plant height (PHT) as distance from the ground level to longest terminal leaf of the plant. Canopy spread (CNS) was taken as the widest ends of the plant, while terminal leaf length (TLL) and terminal leaf width (TLW) were measured were measured as the distance from the leaf tip to the point the leaf by the leaf blade ends on the leaf stalk and the widest ends across the leaf blade, respectively. Seed length (SDL), seed width (SDW), and seed height (SHT) were determined using a Digital Vernier Calipers (cm) on ten randomly, but well developed and uniform seeds. SDL and SDW were measured as the height of the longest and the widest sides of the seed respectively, while SHT was taken as the height between the hilum and the dorsal end of the seed. Means and ranks were computed. The qualitative data recorded included pod shape and colour, seed shape, seed coat colour and presence and absence of a seed eye determined by visual assessment, and seed texture was determined visually and most frequently by hand feeling. Leaf morphology was evaluated through visual observation. All data recorded were according to descriptors for Bambara groundnut (IPGRI/IITA/BAMNET, 2000) with some modifications; and records were averaged.

4.2.3 Data analysis

All the quantitative traits over the two replications were computed for all landraces over the seven incomplete blocks and subjected to analysis of variance (ANOVA) based on the lattice procedure, using Agrobase (Agrobase, 2005) and the SAS statistical program (SAS, 2002). Treatments' means were separated by the least significant differences (LSD) at 5% probability. Descriptive statistics was employed to analyze qualitative data using percentages (%).

Table 4.1 List of landraces and their origin used in the study

S/no.	Accessions	Origin	Entry status	S/no.	Accessions	Origin	Entry status
1	211-31	CAPS	2011 entry	26	211-75	CAPS	2011 entry
2	211-45	CAPS	2011 entry	27	211-76	CAPS	2011 entry
3	211-46	CAPS	2011 entry	28	211-77	CAPS	2011 entry
4	211-47	CAPS	2011 entry	29	211-79	CAPS	2011 entry
5	211-48	CAPS	2011 entry	30	211-80	CAPS	2011 entry
6	211-52	CAPS	2011 entry	31	211-82	CAPS	2011 entry
7	211-53	CAPS	2011 entry	32	211-83	CAPS	2011 entry
8	211-55	CAPS	2011 entry	33	211-84	CAPS	2011 entry
9	211-56	CAPS	2011 entry	34	211-85	CAPS	2011 entry
10	211-57	CAPS	2011 entry	35	211-86	CAPS	2011 entry
11	211-58	CAPS	2011 entry	36	25-1	ZM	ZM 5425
12	211-59	CAPS	2011 entry	37	32-1	ZM	ZM 3236
13	211-60	CAPS	2011 entry	38	42-2	ZM	ZM 2042
14	211-61	CAPS	2011 entry	39	89-1	ZM	ZM 5689
15	211-62	CAPS	2011 entry	40	KB 08	ARC	KUBU 08
16	211-63	CAPS	2011 entry	41	KN 211-6	KNG	2011 entry
17	211-64	CAPS	2011 entry	42	KN 211-7	KNG	2011 entry
18	211-65	CAPS	2011 entry	43	KN 211K	KNG	2011 entry
19	211-66	CAPS	2011 entry	44	M08-1	ZIM	ZIM 108
20	211-67	CAPS	2011 entry	45	M09-3	ZIM	ZIM 109
21	211-68	CAPS	2011 entry	46	SB 19-3-1	ARC	SB 19-3-1
22	211-69	CAPS	2011 entry	47	TV-14	IITA (Ghana)	TVSu 1466
23	211-71	CAPS	2012 entry	48	TV-39	IITA (Sudan)	TVSu 390
24	211-72	CAPS	2011 entry	49	TV-93	IITA (Kenya)	TVSu 793
25	211-74	CAPS	2011 entry				

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

4.3 Results and discussions

There were significant ($P<0.05$) variations in some of the agronomic traits including days to 1st seedling emergence, days to 50% seedling emergence and canopy spread, among the Bambara groundnut landraces. Terminal leaf width were highly ($P<0.001$) significant, and there was no significant variation for plant height and terminal leaf length (Table 4.2). Among the three seed traits evaluated, seed length was ($P<0.01$) significant, whereas seed height showed significance at $P<0.05$, there was no variation among the genotypes for. The extent of variations observed calls for plant selection that can further be evaluated for the confirmation of homogeneity. Also, significant ($P<0.05$) differences were detected for all the

aforementioned traits between the replicates, probably due to variations of heterogeneity in the soils of the experimental field.

Mean values for days for 1st and 50% seedling emergence ranged from 9 to 13.5 days for KN 21-7 and 211-31, and 11 to 22 for 42-2 and 211-47, respectively (Table 4.3). This corroborates with reports of characterization of Bambara groundnut landrace in Burkina Faso that reported germination of 83.0% at 14 days after planting (DAP) (Ouedraogo *et al*., 2008), while a range of 14 to 27 DAP and a mean of 21 DAP for 64.0% germination were reported by Abu and Buah (2011). The mean plant height ranged from 19.7 to 27.9 cm for TV-14 and 211-86, while canopy spread was 28.4cm to 52.0cm for 211-48 and 211-86, in that order. Canopy spread with a range of 22.0cm to 47.0cm was reported in Ghana (Abu and Buah, 2011). Terminal leaf length measured from 5.3cm to 7.8cm for 211-79 and 211-72, while terminal leaf was 1.8cm to 3.35cm for 211-86 and 211-75, respectively. Seed length (measured as the longest ends of the seed) and width (measured as the distance between the sides of the seed with the seed eye facing up) were measured at 8.6mm to 13.1mm for KB 08 and TV-39, and 7.6mm to 10.1mm for 211-86 and 89-1. Seed height (measured from the seed eye to the dorsal part of the seed) ranged from 7.4mm to 10.0mm for landraces KB 08 and TV-93. Significant ($P<0.05$) difference have been reported for some quantitative traits, such as plant spread, plant height, seed length and seed width (Ntundu *et al*., 2006). Shegro *et al*. (2013) opined that cultivar and environment influence morphological dimensions among Bambara groundnut landraces.

Table 4.2 Summary statistics of mean square and significant differences of agronomic and seed traits among 49 Bambara groundnut landraces tested using the partially unbalanced lattice design with seven incomplete blocks and two replications

		SEM		FPEM		PHT		CNS		TLL		TLW	
Source of variation	Df	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replication	1	8.582	5.21*	32.0	6.96*	63.362	15.33*	415.955	17.87*	2.984	12.64*	0.444	8.04*
Genotype (Unadjusted)	48	2.125		8.751		5.926		43.969		0.342		0.217	
Block (Adjusted)	12	0.410		2.905		1.329		7.390		0.936		0.020	
RCBD (Residual)	48	1.957		5.021		4.835		27.242		0.276		0.064	
Genotype (Adjusted)	48	2.125	1.09*	8.751	1.74*	5.926	1.23NS	43.969	1.61*	0.342	1.24NS	0.217	3.39**

		SDL		SDW		SDH	
Source of variation	Df	MS	F-value	MS	F-value	MS	F-value
Replication	1	2.880	7.23*	0.059	0.12NS	1.569	6.64*
Genotype (Unadjusted)	48	1.516		0.733		0.565	
Block (Adjusted)	12	0.120		0.106		0.064	
RCBD (Residual)	48	0.468		0.599		0.279	
Genotype (Adjusted)	48	1.516	3.24**	0.733	1.22NS	0.565	2.02*

SEM (Days to 1st seedling emergence); **FPEM** (Days to 50% seedling emergence); **PHT** (Plant height); **CNS** (Canopy spread); **TLL** (Terminal leaf length); **TLW** (Terminal leaf width); **SDL** (Seed length); **SDW** (Seed width); **SDH** (Seed height); *Significant difference at the 0.05 probability level; **Significant difference at the 0.001 probability level; **Df** (Degree of freedom); **MS** (Mean square); **NS** (Not significant)

Table 4.3 Mean response and ranks of agronomic and seed traits among 49 Bambara groundnut landraces

		DTEM		FPEM		PHT		CNS		TLL		TLW		SDL		SDW		SDH	
S/No.	Landraces	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	211-31	13.5	1	20.0	2	22.8	29	41.8	22	6.4	45	2.5	38	11.35	13	9.8	5	9.1	6
2	211-45	12.5	4	16.5	14	24.15	12	44.15	10	7.45	3	3.1	4	11.35	15	9.55	9	9.05	7
3	211-46	11.0	28	15.5	21	23.8	17	40.2	30	7.15	10	2.8	14	10.2	38	8.6	34	8.1	41
4	211-47	12.5	5	22.0	1	23.6	19	43.6	13	7.5	2	3.0	7	10.45	34	8.7	29	8.35	35
5	211-48	10.0	44	15.0	24	19.7	48	28.4	49	6.65	30	2.7	17	10.6	31	8.5	41	8.4	33
6	211-52	11.0	26	15.0	27	23.55	20	36.8	44	7.05	14	2.6	33	11.6	7	8.65	30	8.65	21
7	211-53	9.0	48	12.0	48	23.9	14	47.35	6	7.35	4	2.25	45	11.4	12	9.05	21	9.05	8
8	211-55	10.0	45	16.5	10	22.7	33	42.55	19	6.95	18	2.35	40	10.5	32	8.5	39	8.1	40
9	211-56	11.0	22	13.5	38	22.4	35	41.2	24	6.55	41	2.55	37	11.55	8	9.75	6	9.55	2
10	211-57	11.5	19	14.5	30	24.15	11	43.6	12	7.1	12	2.65	30	9.7	45	8.85	26	8.4	29
11	211-58	9.5	47	13.5	37	21.9	37	39.9	34	6.6	38	2.6	32	11.35	14	8.9	24	8.65	20
12	211-59	10.5	30	15.5	20	25.15	6	43.15	14	6.75	26	3.25	2	10.5	33	8.55	35	8.0	42
13	211-60	10.5	31	15.0	23	25.7	3	41.8	21	7.25	7	2.9	8	10.15	40	7.95	45	8.25	36
14	211-61	10.5	33	13.5	39	20.6	47	42.6	18	6.7	29	2.7	19	10.7	27	8.1	44	8.95	12
15	211-62	11.0	23	14.5	28	23.25	24	39.0	37	6.75	28	2.7	18	10.75	25	8.95	22	8.75	15
16	211-63	10.5	37	13.5	41	23.2	26	40.0	32	6.55	39	2.9	9	11.7	5	9.2	16	8.95	10
17	211-64	10.0	39	13.5	42	20.65	46	38.7	39	6.6	37	2.7	24	10.9	22	9.15	18	8.7	18
18	211-65	10.0	43	16.5	12	21.3	44	42.9	17	6.3	46	2.15	46	10.3	37	8.7	28	8.5	27
19	211-66	11.0	24	16.0	16	23.95	13	49.45	3	6.9	19	2.8	15	12.05	4	9.45	11	8.95	9
20	211-67	12.0	9	15.5	19	23.4	21	40.0	33	6.6	35	2.35	39	9.6	46	9.1	19	7.65	46
21	211-68	12.0	12	13.5	43	22.75	30	36.65	45	6.6	36	2.3	42	10.65	29	8.5	37	8.15	37
22	211-69	11.5	15	15.0	22	23.3	23	38.3	41	6.65	32	2.6	36	10.15	39	8.5	40	7.9	43
23	211-71	12.0	10	13.0	46	27.4	2	48.75	5	6.6	34	2.35	41	10.05	42	8.55	36	8.15	38
24	211-72	10.5	36	16.5	13	21.45	43	41.8	20	7.8	1	1.9	47	10.6	30	9.15	17	8.4	31
25	211-74	11.0	25	14.0	35	22.1	36	40.9	26	6.8	25	2.85	11	10.8	24	9.25	15	8.4	30
26	211-75	10.0	42	13.5	40	24.6	9	42.95	16	7.25	8	3.35	1	11.45	11	8.6	32	8.7	17
27	211-76	10.0	40	15.0	25	24.8	8	40.9	27	6.85	24	2.85	10	9.75	44	7.85	48	7.65	47

Table 4.3 Continued

		DTEM		FPEM		PHT		CNS		TLL		TLW		SDL		SDW		SDH	
S/No.	Landraces	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
29	211-79	10.5	32	16.0	15	23.25	25	46.5	7	5.3	49	3.05	6	10.95	20	9.8	4	8.5	28
30	211-80	11.0	21	15.5	18	23.3	22	43.8	11	6.85	23	2.7	23	11.25	16	8.85	25	8.85	14
31	211-82	10.5	34	18.5	4	22.75	32	41.2	25	6.75	27	2.65	31	10.65	28	8.5	38	8.4	32
32	211-83	12.5	6	14.0	33	23.1	27	39.8	35	6.9	21	2.75	16	9.6	47	7.9	47	7.8	44
33	211-84	11.5	18	17.0	9	23.1	28	40.6	29	6.9	22	2.65	29	11.5	9	9.35	12	8.95	11
34	211-85	10.0	46	17.0	8	23.8	18	44.2	9	7.0	17	2.85	13	10.7	26	9.25	14	8.6	24
35	211-86	11.0	29	14.5	29	27.9	1	52.0	1	7.1	11	1.8	49	8.9	48	7.55	49	7.45	48
36	25-1	12.5	7	17.5	6	24.2	10	50.3	2	6.5	42	2.7	27	11.5	10	8.8	27	8.75	16
37	32-1	11.0	20	18.0	5	23.9	15	49.25	4	7.3	6	3.15	3	11.15	18	9.35	13	8.65	23
38	42-2	10.0	41	11.0	49	25.5	4	38.7	38	6.5	43	2.6	35	10.95	21	9.95	2	8.85	13
39	89-1	11.5	17	14.0	34	21.55	42	44.65	8	6.45	44	2.85	12	11.25	17	10.1	1	9.15	5
40	KB 08	13.0	2	17.0	7	20.65	45	43.15	15	7.05	13	2.7	25	8.6	49	8.1	43	7.35	49
41	KN 211-6	12.0	8	15.0	26	21.65	39	31.25	47	6.3	47	2.3	43	9.8	43	8.4	42	8.1	39
42	KN 211-7	9.0	49	13.0	44	23.8	16	40.0	31	7.0	16	1.8	48	10.4	35	9.05	20	8.5	26
43	KN 211K	12.0	11	14.5	32	21.55	41	30.05	48	6.65	31	2.7	22	11.7	6	9.6	8	9.2	4
44	M08-1	11.5	14	14.0	36	22.75	31	37.6	43	6.65	33	2.65	28	10.85	23	8.9	23	8.65	22
45	M09-3	10.5	35	14.5	31	21.85	38	38.3	40	6.9	20	2.7	26	10.1	41	7.95	46	7.7	45
46	SB 19-3-1	13.0	3	15.5	17	22.45	34	41.55	23	7.25	9	2.6	34	10.4	36	8.65	31	8.35	34
47	TV-14	11.0	27	13.0	45	19.65	49	35.15	46	6.05	48	2.3	44	11.0	19	8.6	33	8.55	25
48	TV-39	10.5	38	12.5	47	25.05	7	38.2	42	7.3	5	2.7	20	12.6	2	9.5	10	9.3	3
49	TV-93	12.0	13	19.5	3	25.4	5	39.3	36	6.55	40	3.05	5	13.1	1	9.85	3	9.95	1
	Mean	11.07		15.22		23.16		41.30		6.82		2.65		10.80		8.90		8.52	
	R^2 (%)	54.1		65.2		60.0		65.9		59.4		77.9		77.1		55.1		68.2	
	CV (%)	12.6		14.7		9.5		12.6		7.7		9.6		6.3		8.7		6.2	
	LDS (0.05)	2.35		3.76		3.69		8.75		0.88		0.42		1.15		1.30		0.89	

SEM (Days to 1st seedling emergence); **FPEM** (Days to 50% seedling emergence); **PHT** (Plant height); **CNS** (Canopy spread); **TLL** (Terminal leaf length); **TLW** (Terminal leaf width); **SDL** (Seed length); **SDW** (Seed width); **SDH** (Seed height)

Table 4.4 Showed the descriptive statistics of pod and seed morphology (shape) among 213 landraces, and that of leaf morphology among 158 Bambara groundnut landraces. There is scant information describing pod and seed morphology in Bambara groundnut landraces. In this study, 102 pods types could be distinguished, with pointed and round pod shape the highest number, 102 had a round shape, and 76 a pointed shape, representing 47% and 35.7%, respectively (Table 4.4 and Fig. 4.1). According to IPGRI/IITA/BAMNET (2000) none of the landraces observed had pods without a point. Only four pod colours were observed (Fig. 4.2). About 76% were yellowish in colour, and only 4.7% had reddish brown pod colour. Within the four descriptors for pod texture, 72% had little grooved texture and <1% of the landraces had a much folded texture (Fig. 4.3). Between the two descriptors used to describe seed shape, 169 accessions were oval and 44 were round, representing 79% and 21%, respectively. Absence and presence of an "eye" (Table 4.4 and Fig. 4.4) were at about 59% for no eye and 41% for an eye being present.

Fifteen descriptors were employed to describe the various types of seed coat colour displayed by the Bambara groundnut landraces. Out of the 213 landraces studied, cream coat colour dominated with 79 landraces, representing 37.1%. This was followed by brown and light brown seed coat colours with 33 landraces, representing 15.5% each for each. The least common coat colours were at <1%, displayed by only one landrace each for eight different seed coat colours. Conversely, 158 landraces were used to define leaf morphology using four descriptors (Table 4.4 and Fig. 4.5) wherein 78 landraces had a round leaf shape (49.4%); elliptic leaves were observed amomg 34 landraces (21.5%). Twenty four accessions showed oval leaves shape (15.0%); and 22 landraces had lanceolate shapes (14%). The counts of types of leaf morphology among 61 Bambara groundnut landraces that were evaluated in the field are presented in Table 4.4. Landrace 211-85, which originated from Capstone Seed Company, had the highest within landrace variation by possessing all the four descriptors of leaf morphology (Table 4.5). Ten landraces had three of the four leaf morphology descriptors with respect to the individual landraces. Twenty two of the landraces had two variants of leaf shape. In general, 27 landraces revealed uniformity by possessing only one type of leaf morphology (44.3%) while 34 landraces were heterogeneous (55.7%), possessing more than one form of leaf shapes. In an evaluation of Bambara groundnut landraces in Burkina Faso, Ouedraogo *et al*. (2008) observed that only 18.0% were homogenous. The findings in this study reflect the necessity for extensive sorting and classification of the Bambara groundnut landraces collected form seven different geographical locations, as presented in the previous chapter. This stresses the need for such classification of Bambara groundnut landraces, in order to provide breeders with homogenous seed materials for scientific breeding projects.

Table 4.4 Pod and seed morphological traits among selected Bambara groundnut landraces and corresponding number and percentage of landraces

Traits	Description	Number of landraces bearing the trait	% number of landraces bearing the respective traits
Pod shape	1. Without point	0	0.0
	2. Point + round	102	47.9
	3. Point + nook	35	16.4
	4. Point + point	76	35.7
Pod colour	1. Yellowish	162	76.1
	2. Brown	28	13.2
	3. Reddish brown	10	4.7
	4. Purple	13	6.1
Pod texture	1. Smooth	42	19.7
	2. Little grooves	154	72.3
	3. Much grooves	16	7.5
	4. Much folded	1	0.5
Seed shape	1. Round	44	20.7
	2. Oval	169	79.3
Seed eye	1. No eye	126	59.2
	2. Present	87	40.9
Seed coat colour	1. Black	11	5.2
	2. Black/purple	1	0.5
	3. Brown	33	15.5
	4. Brown speckle	5	2.5
	5. Brown with spots	1	0.5
	6. Cream	79	37.1
	7. Cream with black stripe	1	0.5
	8. Cream *RBF* eye	2	0.9
	9. Cream stripe	1	0.5
	10. Cream variegated	1	0.5
	11. Cream/purple	1	0.5
	12. D/brown	14	6.6
	13. D/brown speckle	10	4.7
	14. D/brown with spots	1	0.5
	15. L/brown	33	15.5
	16. L/brown speckle	4	1.9
	17. L/brown with spots	1	0.5
	18. Red	9	4.2
	19. Tan	5	2.4
Terminal leaf shape*	1. Round	78	49.4
	2. Oval	24	15.2
	3. Elliptic	34	21.5
	4. Lanceolate	22	13.9

*Assessed among 158 landraces

Fig. 4.1 Bambara groundnut landraces assorted by pod shape: A point + round; B point + nook; and C point + point

Fig. 4.2 Bambara groundnut landraces assorted by dry pod colour: top left (yellowish); top right (purple); bottom left (brown); and bottom right (reddish brown)

Fig. 4.3 Bambara groundnut landraces assorted by dry pod texture: A smooth; B little grooves; C much grooves; and D much folded

Fig. 4.4 Description of the presence and absence of eye on the seeds of Bambara groundnut landraces: top 1-3 (Showing presence of eye); and bottom 1-3 (Absence of eye)

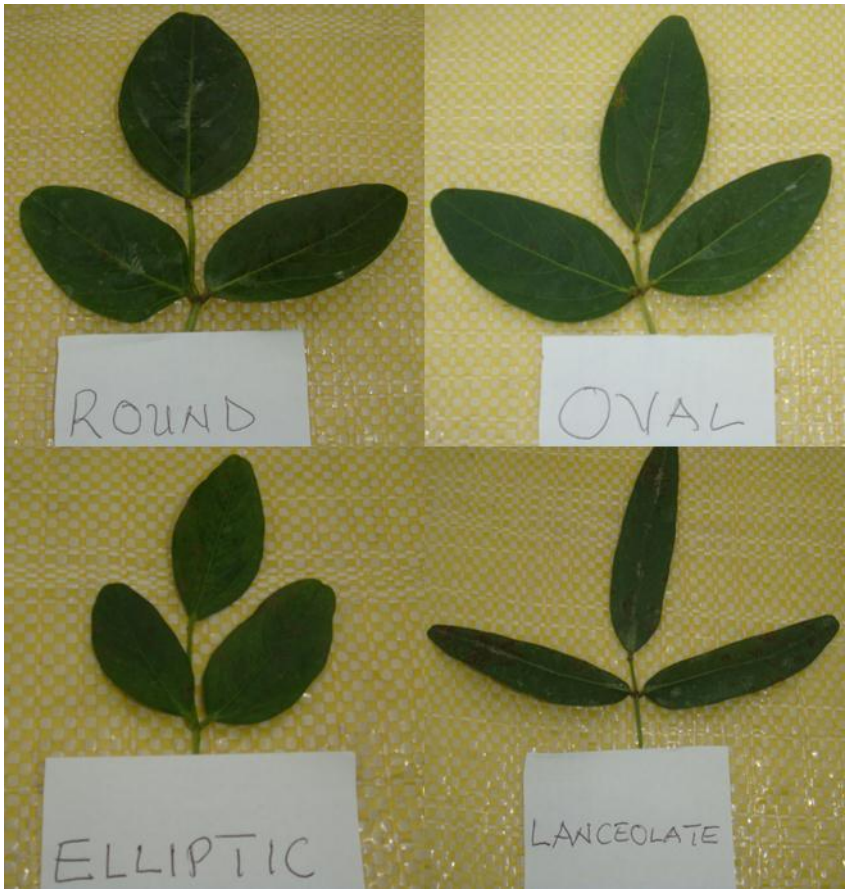

Fig. 4.5 Description of leaf morphology among Bambara groundnut landraces: top left (Round); top right (Oval); bottom left (Elliptic); and bottom right (Lanceolate)

Table 4.5 Summary of count of types of leaf morphology (shape) among 61 Bambara groundnut landraces evaluated in the field

Name of landrace	Type of leaf shape*	Number observed	Name of landrace	Type of leaf shape	Number observed	Name of landrace	Type of leaf shape	Number observed	Name of landrace	Type of leaf shape	Number observed
211-30	1,3,4	3	211-63	1	1	211-79	1,2	2	KN 211-1	1	1
211-31	1,3,4	3	211-64	1	1	211-80	1,3	2	KN 211-13	1	1
211-45	1,2	2	211-65	1,3	2	211-81	2	1	KN 211-2	1	1
211-46	1	1	211-66	1,2,4	3	211-82	1	1	KN 211-6	1,4	2
211-47	1,2	2	211-67	1,3	2	211-83	1	1	KN 211-7	3	1
211-48	1,3	2	211-68	1,2,3	3	211-84	1,4	2	KN 211-8	4	1
211-52	1,4	2	211-69	1,4	2	211-85	1,2,3,4	4	KN 211K	4	1
211-53	1,3	2	211-70	1,2	2	211-86	2,3	2	M08-1	1,2,3	3
211-55	1,3	2	211-71	1,2,3	3	211-96	1,3	2	M09-3	1,2,3	3
211-56	1,2,3	3	211-72	1,3	2	211-98	1	1	SB 19-3-1	1,3	2
211-57	1,3	2	211-73	1	1	25-1	1,4	2	TV-14	1	1
211-58	1,3	2	211-74	1	1	32-1	2,4	2	TV-27	4	1
211-59	2	1	211-75	2	1	42-2	1	1	TV-39	1	1
211-60	1,4	2	211-76	1	1	89-1	2	1	TV-93	1	1
211-61	1,2,4	3	211-77	2,3,4	3	KB 08	1	1			
211-62	1	1	211-78	1	1						

* See Table 4.4 for terminal leaf shapes descriptions

4.4 Conclusion

The findings in this study established the presence of sufficient within- and between-variations among the Bambara groundnut landraces for scientific breeding to be undertaken. This is owing to the existence of several morpho-types within the landraces. Results of the current study corroborates with those of Ntundu *et al*., (2006) who showed the presence of variation for agronomic and seed characters among Bambara groundnut landraces in their study. The need remains for systematic selection and breeding of Bambara groundnut landraces to boost productivity and yield stability in this crop.

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CHAPTER FIVE

Morphological characterization and evaluation of Bambara groundnut genotypes for yield and yield related traits

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is an important, but under-utilized legume crop grown in sub-Saharan Africa mostly by resource poor farmers. Landraces of the crop whose genetic diversity has not been evaluated were grown in the field. The objective of this study was to characterize and evaluate yield and yield component response of 49 genotypes of Bambara groundnut derived from single plant selections of diverse germplasm collections. Field evaluations were conducted involving 26 yield and yield related traits, using a partially balanced lattice design with three replications. Highly significant (P<0.001) differences were detected among the genotypes for canopy spread, petiole length, total biomass, seed weight and seed height, while seedling emergence, pod weight, seed length and seed width were significantly different (P<0.05). Principal component (PC) analysis identified nine influential components, of which two components, PC_1 and PC_2, highly contributed to the total variation at 19% and 14%, respectively. Leaf colour at emergence, petiole colour, leaf joint pigmentation and calyx colour were highly correlated with PC_1, while seed length, seed width and seed height had strong association with PC2. Both the principal component and cluster analyses showed that most genotypes associated with one another with respect to agronomic and seed yield traits irrespective of geographical location. Among the genotypes, 211-57, MO9-4 and TV-27 showed high seed yield performances, while TV-93 and 45-2 had higher biomass production were selected for their respective agronomic performances. These selected true-to-type genotypes can be used for direct large-scale production, breeding or germplasm conservation.

Keywords: Bambara groundnuts, cluster analysis, genotype, landraces, principal component analysis, true-to-type.

5.1 Introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc.; **Syn:** *Voandzeia subterranea* [L.] Thouars.) is an under-utilized grain legume grown in sub-Saharan Africa (SSA), mostly by women as a source of protein for subsistence (Ntundu *et al*., 2004). It is a member of the family Fabaceae, and subfamily Papilionoideae. The crop is commonly referred to as a poor man's crop and has thus far received little research focus by the scientific world. Bambara groundnut is third in importance in SSA among grain legumes after groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* [L.] Walp.) (Linnemann and Azam-Ali, 1993).

Bambara groundnut grains make up a complete balanced food (Rowland, 1993). The major proportion of the diet of the rural and urban communities in Africa consists of starchy foods such as sorghum, maize and millet. Therefore, Bambara groundnut cultivation in SSA supplements and diversifies the starch nutrition, improving the nutritional intake of millions of Africans. Bambara groundnut is primarily cultivated for its pod-borne seeds. The seeds are rich in protein (16-25%), carbohydrates (~ 63%) and oil (~18%) which is composed of various fatty acids. The predominant fatty acids include oleic acid, palmitic acid and linolenic acid (Minka and Bruneteau, 2000). Chemical analyses showed that it contains 32.50-32.72% of total essential amino acids including lysine, histidine, arginine, leucine and isoleucine, and 66.10-70.80% of total non-essential amino acids such as methionine, glycine, cysteine, tyrosine and proline (Minka and Bruneteau, 2000; Amarteifio *et al*., 2006; Aremu *et al*., 2006).

Various parts of Bambara groundnut are used for human consumption, the young fresh seeds may be boiled and eaten as a snack in a manner similar to boiled peanut, and dry seeds can be made into pudding (or steamed-paste) called Moi-Moi or Okpa (bean porridge) in some parts of Nigeria (Okpuzor *et al*., 2009). As a vegetable, pods are harvested at an immature stage, boiled in which the inner seed are eaten during 'Hunger Gap', an interim period during the growing season when there is little food among rural families and main crops are not ready for harvest.

The crop has the potential of producing greater than 3,000 kg ha^{-1} of seed yield (Collinson *et al*., 2000). Larger part of the annual production of Bambara groundnut comes from West Africa at 45-50% of this region; Nigeria leads in its production (Goli *et al.*, 1997). Bambara groundnut is drought tolerant and has the ability to fix atmospheric nitrogen through symbiosis with the bacterium, *Bradyrhizobium* sp., borne in the root nodules of the lateral roots. In general, Bambara groundnut has the potential to enhance nutritional security for humans (Massawe *et al*., 2002).

Despite its many advantages, little research has been conducted on Bambara groundnut. Its potential has been neglected relative to cash crops that possess marketing and industrial benefits, such as sugarcane, cocoa, coffee, cotton, and other durable, transportable, and commercially valuable crops and their by-products such as peanut and its oil (Massawe *et al*., 2005). To date, the genetic potential of Bambara groundnut remains largely unexploited. Thus far, only farm level selection is being practiced in which existing landraces are evaluated and their seeds multiplied for use by farmers. As such there are no improved varieties of Bambara groundnut available in the major growing areas. The existing landraces can provide breeders with sources of genes for biotic and abiotic resistances, adaptability to different environments, nutritional characteristics and yield potential. The diversity of Bambara groundnut landraces reflects the absence of any active breeding work.

Germplasm of Bambara groundnut collection, which comprise of 58 seed lots were obtained from seven diverse geographic origins in SSA. The accessions were phenotyped using seed morphology, including seed coat colours and patterns, seed eye colours and patterns, and seed hilum and colours and patterns. Using this approach, 353 different seed morpho-types of the crop were isolated. These accessions were further evaluated for inter- and intra- genetic diversity from which single plant selection was carried out based on defined morphological, agronomic and seed traits. These aided selection for pure and homogenous lines for use in breeding. Morphological and molecular markers and pedigree analyses are widely used in germplasm characterization, and to establish genetic diversity and relationships that may exist in crop plants (Ntundu *et al*., 2006; Olukolu *et al*., 2012). Morphological traits are among the earliest markers, and are still used in germplasm characterization and management (Ntundu *et al*., 2006). The objective of this study was to characterize 49 genotypes of Bambara groundnut using 26 morphological traits, and to evaluate their response to yield and yield components. All the genotypes were derived from single plant selections made from a diverse germplasm collection.

5.2 Materials and methods

5.2.1 Plant material

Forty nine genotypes of Bambara groundnut landraces were used in this study. The genotypes consisted of single plant selection from an initial collection of landraces which were characterized for their seed morphological and inter- and intra-genetic diversity. The selected genotypes represent landrace collections from seven geographical zones in the sub-Saharan Africa (Table 5.1).

5.2.2 Study site

The experiment was conducted from December, 2012-April, 2013 at the Research and Training Farm of the University of KwaZulu-Natal, Pietermaritzburg, at Ukulinga, South Africa. The site is situated on a Latitude 30^{o} 24'S, Longitude 29^{o} 24'E, and is 800m above sea level. The soil pH was 4-5, clay percentage 34%-38%, organic carbon 2.5%-3.2% and organic N 0.36%. Relative humidity varied between 30%-100% throughout the season, with temperatures varying between 20-30°C, and 322 mm of rain. (Source: University of KwaZulu-Natal weather station).

5.2.3 Experimental design, field management and data collection

The Bambara groundnut genotypes were evaluated using a partially balanced lattice design with three replications. The genotypes were randomised to the seven incomplete blocks across the three replications. The experimental plot comprised of three rows measuring 2.2m x 3.0m, with inter and intra row spacing of 0.4 m x 1.0 m. This spacing was proposed to allow the crop to express its production potential. Sowing was done into a flat seedbed, with one seed sown to each stand. Seeds the fail to germinate were replaced

within two weeks after sowing. All relevant agronomic practices were carried out to maintain a healthy crop.

Data on the 26 morphological traits were generated from five plants selected from the central row of each plot within the incomplete blocks over the three replicates. Both qualitative and quantitative data were recorded. The quantitative field data included number of days to 50% seedling emergence (SDE) by counting number of days from planting to 50% seedling emergence. Plant height (PHT) was measured using a measuring ruler and expressed in cm as distance from the ground level to longest terminal leaf of the plant. Canopy spread (CNS) was taken as the widest ends of the plant; terminal leaf length (TLL), terminal leaf width (TLW) were measured were measured as the distance from the leaf tip to the point the leaf by the leaf blade ends on the leaf stalk and the widest ends across the leaf blade, respectively. Petiole length (PETL) was taken between the point of attachment to the stem and the leaf blade. These records were taken from 10 weeks after planting. Qualitative data recorded included leaf colour at emergence (LCE), terminal leave shape (TLS), growth habit (GH), stem pigmentation (SPG), petiole colour (PCL), leaflet joint pigmentation (this is the pigmentation at the point of attachment of petiole to the petiole) (LJP), calyx colour (CCL), fresh pod colour (FPC), pod shape (PSP), dry pod colour (PCL), pod texture (PTX), seed shape (SSP) and seed eye pattern (SEY). The qualitative data were determined by visual observations at 8-10 weeks after planting.

Post-harvest quantitative data were taken two months after harvest by which time all the seeds in the pods were dry. They include dry biomass (BMA), pod weight (PDW), seed weight (SDW) recorded in grams (g) using an OHAUS Precision Standard Measuring Scale, while hundred (100) seed weight (HSW) was recorded also in grams using a more sensitive Mettler Scale. Seed length (SDL), seed width (SDW), and seed height (SHT) were determined using a Digital Vernier calipers (cm) on ten randomly, but well developed and uniform seeds taken from seeds used for 100 seed weight measurement for each of the accessions. Threshing of samples was done manually in preparation for the next post-harvest measurements, which include qualitative data on kernel shape (PDS), kernel colour (PDC), kernel texture (PDT), seed shape (SDS) and seed eye pattern (SEY). These measurements were recorded based on visual observations. All data were recorded according to descriptors for Bambara groundnuts (IPGRI/IITA/BAMNET, 2000) with some modifications.

5.2.4 Data analysis

All the quantitative traits over the three replications were computed for all accessions over the seven incomplete blocks and subjected to analysis of variance (ANOVA), based on the lattice procedure using Agrobase statistical software (Agrobase, 2005). Treatments' means were separated by the least significant differences (LSD) at 5% probability. Cluster and Principal Component Analyses were conducted to

determine similarities and dissimilarities among the genotypes using SPSS (SPSS, IBM Statistics 20). A similarity matrix was used and a dendrogram constructed to describe similarities and differences among the Bambara groundnut genotypes.

Table 5.1 A list of sources of Bambara groundnut accessions used in the study

S/No.	Genotype	Origin	Seed coat colour	Entry status	S/No.	Genotype	Origin	Seed coat colour	Entry status
1	211-77	CAPS	cream	2011 entry	26	211-75	CAPS	Cream	2011 entry
2	211-87	CAPS	black	2011 entry	27	211-46-3	CAPS	Red	2011 entry
3	211-55	CAPS	red	2011 entry	28	211-83-2	CAPS	Cream	2011 entry
4	32-1-1	ZM	light brown	ZM 3236	29	712-4	ZM	Tan	ZM 5712
5	45-2	ZM	tan	ZM 2045	30	N211-1	KNG	Cream	2011 entry
6	211-55-1	CAPS	red	2011 entry	31	KB 05	ARC	Cream	KUBU
7	TV-79-1	IITA (Kenya)*	cream	TVSu 792	32	211-68	CAPS	Cream	2011 entry
8	211-90	CAPS	black	2011 entry	33	101-2	ZM	Cream stripe	ZM 5101
9	211-51	CAPS	red	2011 entry	34	KB 08	ARC	Cream *RBF***	KUBU
10	211-91	CAPS	light brown	2011 entry	35	M12-1	ZIM	Cream	ZIM 112
11	42-2-3	ZM	light brown	ZM 2042	36	712-7	ZM	Tan	ZM 5712
12	84-2	ZM	red	ZM 5684	37	211-45	CAPS	Red	2011 entry
13	N211K	KNG	cream	2011 entry	38	101-2-1	ZM	Cream stripe	ZM 5101
14	73-3	ZM	red	ZM 4673	39	42-2	ZM	Light brown	ZM 2042
15	211-76	CAPS	cream	2011 entry	40	M01-8	ZIM	Cream *RBF*	ZIM 101
16	25-1	ZM	light brown	ZM 5425	41	TV-93	IITA (Kenya)	Cream	TVSu 793
17	B71-2	ARC	cream	SB 7-1	42	M02-3	ZIM	Cream *RBF*	ZIM 102
18	M09-4	ZIM	cream	ZIM 109	43	B71-1	ARC	Cream	SB 7-1
19	N212-5	KNG	brown	2011 entry	44	73-2	ZM	Red	ZM 4273
20	TV-27	IITA (Nigeria)	dark brown speckle	TVSu 275	45	211-88	CAPS	Black	2011 entry
21	M09-3-1	ZIM	cream	ZIM 109	46	N212-4	KNG	Brown	2012 entry
22	011-7	PMB	cream stripe	2011 entry	47	TV-39	IITA (Sudan)	Dark brown speckle	TVSu 390
23	N212-8	KNG	brown	2012 entry	48	211-69	CAPS	Cream	2011 entry
24	211-57	CAPS	red	2011 entry	49	M09-3	ZIM	Cream	ZIM 109
25	42-1	ZM	light brown	ZM 2042					

CAPS= CAPSTONE Seed Company, South Africa; ZM= Zambian National Program; IITA= International Institute of Tropical Agriculture in Ibadan, Nigeria; with a place origin; KNG= Kano, Nigeria; ZIM= Zimbabwean National Program; PMB= Pietermaritzburg; ARC=Agricultural Research Council of South Africa; *RB*F=Red butterfly eye

5.3 Results and discussions

The Bambara groundnut genotypes exhibited considerable variation among the agronomic, as well as seed traits (Table 5.2). Highly significant (P<0.001) differences were detected for canopy spread, petiole length, weight of biomass, seed weight and seed height, while number of days to seedling emergence, pod weight, seed length and seed width were significantly (P<0.05) different (Table 5.2). Table 5.3 summarises the mean responses of agronomic traits among 49 Bambara groundnut genotypes. The mean canopy spread varied from 46.93 to 69.40 cm for genotypes 211-76 and 45-2, respectively; while terminal leaf length and terminal leaf width varied from 5.40 to 8.53 cm for TV-27 and 101-2, and 2.47 to 5.27 cm for 211-69 and 84-2, respectively. Petiole length and weight of biomass varied from 19.50 to 36.17 cm for genotypes TV-14 and 102-1, and 58.8 to 180.40 g, for TV-14 and 45-2, respectively. In a similar diversity study of Bambara groundnut landraces in Tanzania, Ntundu *et al.* (2006), reported significant differences among quantitative traits including petiole length, plant spread, plant height, seed length and seed width, among others. In addition, variation in yield related traits have been reported by Shegro e*t al.* (2013), who showed that cultivar and environment may influence performance. These reports suggested that agronomic and seed traits are useful for the characterization of Bambara groundnut and selection of genotypes suitable for breeding.

Table 5.2 Summary statistics of mean square and significant differences of agronomic, and pod and seed traits among 49 Bambara groundnut genotypes tested using the partially unbalanced lattice design with seven incomplete blocks, and three replications

		SEM		PHT		CNS		TLL		TLW		PETL		BMS	
Source of variation	Df	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replication	2	27.456	6.86*	122.76	15.20**	371.1	16.48**	149.33	82.14**	104.62	67.33**	166.71	18.62**	34126.9	78.00**
Genotype (Unadjusted)	48	6.545		12.27		64.21		2.02		1.76		26.49		1823.93	
Block (Adjusted)	18	2.807		5.15		12.07		0.61		0.38		6.6		183.39	
RCBD (Residual)	96	4.227		8.3		24.48		2.05		1.78		9.4		485.19	
Genotype (Adjusted)	48	6.545	1.55*	2.27	1.42NS	64.21	2.62**	2.02	0.99NS	1.76	0.99NS	26.49	2.82**	1823.93	3.76**

Source of variation		PWT		SWT		HSW		SDL		SDW		SHT	
	Df	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replication	2	12.44	3.61*	2671.4	18.74**	42.74	0.95NS	0.82	1.82NS	0.22	0.66NS	0.22	1.28NS
Genotype (Unadjusted)	48	8.25		444.9		69.03		1.21		0.6		0.47	
Block (Adjusted)	18	2.5		81.22		34.93		0.14		0.23		0.13	
RCBD (Residual)	96	3.62		154.04		46.91		0.51		0.35		0.18	
Genotype (Adjusted)	48	8.25	2.28*	444.9	2.89**	69.03	1.47NS	1.21	2.37*	0.6	1.73*	0.47	2.56**

SEM (Days to seedling emergence); **PHT** (plant height); **CNS** (canopy spread); **TLL** (terminal leaf length); **TLW** (terminal leaf width); **PETL** (petiole length); **BMS** (weight of biomass); **PWT** (pod weight); **SWT** (seed weight); **HSW** (hundred seed weight); **SDL** (seed length); **SDW** (seed width); **SHT** (seed height); *Significant difference at the 0.05 probability level; ** Significant difference at the 0.01 probability level; **Df** (degree of freedom); **MS** (mean square); **NS** (not significant)

Mean values of pod and seed weight per plant varied from 26.5 and 51.33 g, with the highest values being for genotypes 211-69 and 211-57, respectively; while the lowest weight for the two traits were at 15.97 and 4.0 g for genotypes TV-14 and N212-5, respectively (Table 5.3). There were no significant differences for plant height, terminal leaf length, terminal leaf width and hundred seed weight (Table 5.2). Non-significant variation for 100 seed weight was found among Bambara groundnut landraces in Tanzania (Ntundu *et al*., 2006). Number of days to 50% seedling emergence (SEM) ranged from 23.00 to 28.33 with a mean of approximately 26 days. Genotypes TV-93, N211K, 211-87, 42-1 and 211-55 emerged within 23 days after planting as the earliest (Table 5.3), while 211-69 and 211-46-3 emerged late at 28.33 days.

An earlier study indicated that poor yield in Bambara groundnut is associated with a low level of germination, which leads to poor crop establishment especially in drier ecologies (Linnemann and Azam-Ali, 1993). Furthermore, the heterogenic nature of the Bambara groundnut landraces may lead to variability in growth and development (Zulu, 1989). Prolonged storage reduces seed germinability and seedling vigour (Mkandawire, 2007). Variations in the rate of seed germination and seedling emergence in Bambara groundnut have been reported to be impacted by temperature (Massawe *et al*., 2003), and water imbibition (Modi, 2013). Since the genotypes used in this study were genetically uniform, variation in germination may have been due to variability in the soil-micro environment and fluctuation of temperatures before seedling emergence. This would mean that selection can be made for prolific germination of Bambara groundnut seed and establishment of seedlings under varying growth temperatures and soil water condition. Although there was no significant difference among the genotypes for plant height (PHT), the range was from 20.20 to 30.33 for M02-3 and TV-79-1, respectively (Table 5.3). Mean plant height ranged from 37.5 to 25.5 (Ahmad, 2013).

In addition, the trait responses were mainly explained by the R^2 values among the Bambara groundnut landraces (Table 5.3). The results indicated a range of R^2 values of 50% to 77% for plant height and total biomass, respectively. These traits had highly significant differences at (P<0.001) for genotype (Table 5.2). The R^2 values for 100 seed weight and seed weight were 43% and 64% as the lowest and highest, respectively (Table 5.3).

Table 5.3 Mean response and ranks of agronomic traits among 49 Bambara groundnut genotypes derived from single plant selection

S/No.	Genotypes	SEM		PHT		CNS		TLL		TLW		PETL		BMS	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	**Rank**
1	011-7	27	13	23.67	32	55.67	36	6.1	36	4.87	6	28.73	21	91.9	28
2	101-2	27	14	28.67	3	65.67	5	8.53	1	3.63	34	35.23	2	122.03	7
3	101-2-1	24.33	42	29.93	2	67.2	3	6.43	30	5.03	3	36.17	1	128.2	5
4	211-45	26.67	20	25.47	15	58.13	26	6.6	24	4.7	15	29.03	18	96.23	26
5	211-46-3	28.33	1	23.67	33	52.73	45	7.73	9	3.33	40	28.27	23	96.7	21
6	211-51	25	36	25.6	14	59.47	19	7.87	8	3.17	43	28.13	27	69.1	45
7	211-55	23.33	45	23.53	35	52.87	44	5.87	45	4.3	28	26.57	38	79.2	39
8	211-57	24.67	39	24.2	26	67.67	2	6.47	26	4.37	26	30.5	13	91.17	29
9	211-68	26	29	26.07	9	61.07	11	8.4	2	3.87	31	28.07	28	65.97	47
10	211-69	28.33	2	26.13	8	59.67	16	8.23	4	2.47	49	33.9	3	101.43	18
11	211-75	24.67	41	23.27	38	55.4	40	7.17	16	3.37	39	28.17	26	85.03	32
12	211-76	25.33	31	22.07	44	46.93	49	6.03	41	4.67	16	26.5	39	83.8	34
13	211-77	24.67	38	24.07	27	61.6	8	6.03	40	4.83	7	32.53	4	95.77	27
14	211-83-2	23.67	44	24.47	24	57.53	29	7.63	11	3.6	35	30.93	10	103.43	17
15	211-87	23.33	47	26.87	4	60.33	13	5.8	47	4.67	17	31.63	8	96.67	22
16	211-88	28	4	24.73	20	55.47	38	6.9	20	4.43	25	27.43	32	70.17	43
17	211-90	26.67	26	23.73	30	53.93	43	6.43	28	4.73	13	28.17	25	72.23	42
18	211-91	27.33	7	25.73	12	58.2	25	8.2	5	3.9	30	29.8	16	76.77	41
19	25-1	27	12	24.3	25	55.3	41	6.97	18	2.77	47	30.3	14	113.7	11
20	32-1-1	27	10	24.47	23	57.13	30	7.43	12	3.27	41	26.63	37	99.9	19
21	42-1	23.33	46	24.87	18	56.67	32	5.97	44	4.83	9	27.63	30	104.63	16
22	42-2	27	15	23.53	34	57.87	28	5.83	46	4.37	27	30.67	11	120.57	8
23	42-2-3	27	17	24.07	28	61.33	10	6.1	37	4.6	19	26.77	36	114.27	10
24	45-2	26.67	18	25.67	13	69.4	1	6.33	33	4.8	11	32.37	5	180.4	1
25	712-4	27.67	6	26.07	10	58	27	6.23	35	4.93	4	31.93	6	147.83	3
26	712-7	28	3	25.73	11	60.87	12	7.7	10	3.5	37	28.8	20	114.5	9
27	73-2	27.33	9	24.47	22	50.8	47	6.93	19	2.8	46	31.77	7	97.5	20
28	73-3	27	16	23.33	37	55.47	37	6.07	39	4.57	20	27.27	33	108.13	14
29	84-2	25	34	25	17	55.4	39	6.37	31	5.27	1	29.27	17	111.2	12
30	B71-1	27	11	23.73	31	56.07	34	7.4	13	3.67	33	27	35	86.63	31

Table 5.3 Continued

S/no.	Genotypes	SEM		PHT		CNS		TLL		TLW		PETL		BMS	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
31	B71-2	26.67	19	23.07	41	59.47	18	6.33	32	4.8	10	26.03	42	84.23	33
32	KB 05	26.67	23	24.67	21	52.47	46	6.03	43	4.5	23	27.67	29	81.97	36
33	KB 08	24.67	37	23.13	40	56.8	31	6.03	42	4.73	14	25.47	45	77.77	40
34	M01-8	27.33	8	25.07	16	58.67	22	8.33	3	2.67	48	29	19	96.27	25
35	M02-3	27.67	5	30.33	1	60	14	7.93	6	4.2	29	28.57	22	81.37	37
36	M09-3	25.67	30	23.87	29	59.4	20	6.43	27	5.03	2	28.23	24	82.47	35
37	M09-3-1	24.67	40	22.07	45	55.87	35	6.07	38	4.5	22	26.03	41	68.43	46
38	M09-4	26.67	25	26.33	6	64.87	6	7	17	4.53	21	31.63	9	96.57	23
39	M12-1	26	28	26.73	5	58.53	23	7.9	7	3.77	32	30.17	15	69.37	44
40	N211-1	25	35	23.2	39	58.4	24	6.87	21	3.2	42	23.57	48	96.47	24
41	N211K	23.33	48	22.8	43	59.53	17	6.7	23	4.73	12	25.97	43	80.07	38
42	N212-4	24	43	21.67	46	56.4	33	6.53	25	4.83	8	25.17	47	62.33	48
43	N212-5	25.33	33	23	42	61.47	9	5.77	48	4.47	24	25.83	44	108.8	13
44	N212-8	26.67	21	23.33	36	55.13	42	6.3	34	4.63	18	27.1	34	105.67	15
45	TV-14	26.67	22	21.53	47	47.7	48	6.83	22	2.87	44	19.5	49	58.8	49
46	TV-27	25.33	32	20.47	48	58.87	21	5.4	49	3.47	38	25.37	46	139.2	4
47	TV-39	26	27	26.13	7	59.87	15	6.43	29	4.9	5	27.6	31	125.4	6
48	TV-79-1	26.67	24	20.2	49	65.73	4	7.33	14	2.87	45	26.43	40	89.87	30
49	TV-93	23	49	24.8	19	63.87	7	7.27	15	3.53	36	30.6	12	154.33	2
	Mean	25.97		24.48		58.3		6.8		4.11		28.57		97.64	
	R^2 (%)	48		50		62		67		63		64		77	
	CV (%)	7.92		12		8.49		21.03		32.39		10.73		22.56	
	LSD (0.05)	2.79		3.98		6.71		1.94		1.81		4.16		29.88	

Table 5.4 Mean response and ranks of pod and seed traits among 49 Bambara groundnut genotypes derived from single plant selection

S/No.	Genotypes	PWT		STW		HSW		SDL		SDW		SHT	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	011-7	20.07	29	33.63	16	23.7	44	11.23	34	9.37	26	8.83	36
2	101-2	23.37	4	40.9	9	33.03	7	12.87	2	9.97	1	9.57	6
3	101-2-1	23.97	2	29.4	20	30.1	13	12.93	1	9.93	2	9.77	1
4	211-45	20.63	21	39.03	12	30.8	12	11.2	36	9.73	10	8.93	31
5	211-46-3	19.07	44	21.9	35	34.47	6	11.57	23	9.47	22	8.53	42
6	211-51	20.37	24	37.7	13	26.1	34	11.53	25	9.47	20	9.03	24
7	211-55	18.77	47	22.53	33	29.13	18	11.3	33	9.6	16	9.1	20
8	211-57	22.67	5	51.53	1	28.5	23	11.73	20	9.47	23	9.33	13
9	211-68	21.07	11	48.77	4	28	26	11.73	19	9.33	27	9.23	16
10	211-69	26.5	1	22.5	34	26.37	32	11.77	18	9.2	32	8.73	40
11	211-75	19.53	40	23.9	29	27.8	27	11.63	22	9.33	28	8.97	29
12	211-76	17.77	48	23.57	32	24.83	42	11.37	30	9.43	24	8.9	32
13	211-77	21.97	7	39.67	11	29.73	15	11.37	31	9.07	36	8.97	28
14	211-83-2	20.67	20	33.27	17	26.77	30	11.77	16	9.7	12	9.4	10
15	211-87	21.43	9	35.6	14	22.47	47	10.97	40	9.03	37	9	26
16	211-88	19.7	31	21.47	36	21.77	48	10.27	47	8.7	43	8.33	47
17	211-90	19.63	32	27.57	23	34.73	5	11.83	15	9.83	5	9.63	2
18	211-91	20.8	17	25.67	28	23.67	45	10.83	42	9.1	34	8.77	38
19	25-1	19.6	33	30.97	19	32.63	8	11.5	28	8.67	45	8.5	43
20	32-1-1	19.53	39	13.8	42	36.57	4	11.53	24	9.07	35	9.07	21
21	42-1	20.63	22	25.93	26	32.53	9	11.93	11	9.93	3	9.6	3
22	42-2	20.67	19	27.77	22	28.37	24	11.93	10	9.67	13	9.37	12
23	42-2-3	23.53	3	10.87	47	17	49	10.67	46	8.5	47	8.3	48
24	45-2	21.23	10	14.8	41	26.27	33	11.23	35	9.73	8	9.23	15
25	712-4	20.87	15	23.57	31	28.97	19	12.53	4	9.2	31	9.47	8
26	712-7	19	46	26.23	24	25.9	35	11.67	21	8.97	38	8.87	33
27	73-2	19.6	34	17.77	39	25.6	37	11.03	37	8.7	42	8.5	44
28	73-3	20.3	27	21.37	37	28.73	22	12	8	9.63	15	9.57	5
29	84-2	19.57	35	40.37	10	24.9	40	10.97	41	9.13	33	8.63	41
30	B71-1	20.1	30	45.17	7	31.9	11	11.9	14	9.93	4	9.47	7

Table 5.4 Continued

S/No.	Genotypes	PWT		SWT		HSW		SDL		SDW		SHT	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
31	B71-2	20.27	28	46.67	5	28.2	25	11.5	27	9.5	19	8.87	34
32	KB 05	19	45	43.5	8	25.2	38	10.73	43	9.4	25	9.23	17
33	KB 08	19.57	36	46.13	6	24.9	41	11.53	26	9.67	14	9.33	14
34	M01-8	20.3	26	27.9	21	28.8	20	12.03	7	9.73	9	9.17	18
35	M02-3	21	13	17.33	40	23.47	46	11.3	32	8.93	39	8.8	37
36	M09-3	20.87	14	23.73	30	27.73	28	11.5	29	9.53	18	9.37	11
37	M09-3-1	19.4	41	33.97	15	25.6	36	11	38	9.27	30	8.93	30
38	M09-4	22.47	6	49.63	2	26.57	31	11.9	13	9.53	17	9.03	25
39	M12-1	20.87	16	25.73	27	32.13	10	12.63	3	9.7	11	9.47	9
40	N211-1	19.07	43	12.5	45	24	43	10.7	45	9.3	29	9.07	22
41	N211K	20.33	25	31.1	18	36.73	3	11.97	9	9.47	21	9	27
42	N212-4	19.57	37	11.23	46	26.77	29	12.47	5	9.8	6	9.6	4
43	N212-5	20.4	23	4	49	43.6	1	10.17	49	8.2	49	8.1	49
44	N212-8	19.53	38	20.8	38	25.13	39	12.17	6	8.87	40	9.1	19
45	TV-14	15.97	49	12.9	44	29.27	17	10.2	48	8.6	46	8.37	46
46	TV-27	19.3	42	49.07	3	29.6	16	10.7	44	8.83	41	8.47	45
47	TV-39	20.73	18	26.17	25	29.83	14	11.77	17	9.77	7	9.07	23
48	TV-79-1	21.03	12	13.63	43	41.33	2	11.9	12	8.7	44	8.87	35
49	TV-93	21.77	8	7.97	48	28.77	21	11	39	8.3	48	8.73	39
	Mean	20.49		28.2		28.53		11.51		9.3		9.02	
	R^2 (%)	55		64		43		55		47		57	
	CV (%)	9.29		44.02		24.01		6.21		6.35		4.74	
	LSD (0.05)	2.58		16.83		9.29		0.97		0.8		0.58	

5.4 Principal component analysis

Results of the principal component analyses (PCA) for the 26 agronomic and seed traits among the 49 Bambara groundnut genotypes are presented in Table 5.5. All 26 traits were grouped under nine components (Eigen values ≥ 1) which accounted for 79% of the variation. The nine principal components (PCs) and corresponding correlation coefficients (or loading values) for all the traits are presented in Table 5.5. Leaf colour at emergence, petiole colour, leaflet joint pigmentation and calyx colour were highly correlated with PC_1, which accounted for 19.7% of the total variation. Seed traits which include seed length, seed width and seed height were correlated with PC_2, while pod weight and weight of biomass correlated with PC_3. Similarly, PC_4 contributed to 8.1% of the available variation and was well correlated with terminal leaf length and plant height. Association between pod weight and biomass, and leaf length and plant height probably explains the efficiency of the transformation of photosynthates into pod and leaf size, which may eventually affect yield. The utilization of agronomic and seed yield traits had been used in a Bambara groundnut improvement program (Shegro *et al*., 2013). It was also observed that, fresh kernel colour correlated well with dry kernel colour, which was found in PC_5 contributing to 7.8% of the variation, suggesting that fresh pod colour may affect colour in dry condition. However, PCs 6, 7 and 8 had high correlations with 100 seed weight, kernel texture and leaf shape, contributing 6.2, 5.3 and 4.6% to the observed variability, respectively. PC_9 contributed to almost 4.0% of the variability in which stem pigmentation was important. In general, the PC analysis of the 26 traits indicated that PC_1 was composed of a number of traits that contributed for the greatest variation, followed by PC_2. In this study, it was observed that Bambara groundnut farmers may have driven the selection for specific morphological and seed traits. A similar observation was made by Ntundu *et al*. (2006) who reported that leaf morphology, seed size and colour were morphological criteria used by farmers in Tanzania during selection.

5.5 Principal component biplot

The wide variation observed among the Bambara groundnut genotypes used in this study were expressed by the PCA biplot (Fig. 5.1). The biplot explained relationships and similarities that exist among the Bambara groundnut genotypes, relative to the 26 measured traits in the study. The genotypes were scattered within the four quadrants produced by the PC_1 and PC_2 biplot. In terms of their genetic variability, the genotypes displayed a pairing orientation, irrespective of geographical locations within the axes, suggesting that they share most of the features for the 26 traits that were studied. This feature of orientation would suggest that movement of Bambara groundnut landraces across the African sub-region was indiscriminate. It further refers that genotypes from common origin paired in the same group. Grouping of Bambara groundnut landraces from the same region in Tanzania was earlier reported (Ntundu *et al*., 2006). Conversely, genotypes that scatter far apart within the axes were distantly related to other landraces within the same quadrant. PC_1 and PC_2 were

the only principal components where they represent 20% and 14% of the total variations, respectively presented in the principal component analyses above.

Results of the biplot showed that landraces 211-83-2, 42-2-3, 211-55, 211-55-1, M09-3, M09-4, 211-68 and N211K (Fig. 5.1A) which originated from Capstone, Zambia, Zimbabwe and Kano, Nigeria, respectively had strong associations. It is probable that these genotypes originated from the same region. Also strongly associated were landraces 211-91, N211-1 and 25-1 (Fig. 5.1B), which originated from CAPS, Kano, Nigeria and Zambia, respectively. KB 05, 211-77 and N212-8 (Fig. 5.1C) from ARC in South Africa, CAPS and Kano in Nigeria, respectively formed another associated group. B71-2 and 712-7 originating from the ARC in South Africa and Zambia, respectively displayed a strong association (Fig. 5.1D). A Strong association was observed between the Bambara groundnut genotypes 42-2 and KB 08 (Fig. 5.1E). Although they are distant from the more densely associated groupings, genotypes 45-2 and TV-14 had a strong association (Fig. 5.1F). A Comparable relationship was shown between genotypes TV-39 and 712-4 (Fig. 5.1G), which originated from Sudan and Zambia, respectively. Conversely, certain genotypes were distantly grouped, including N212-4 and N212-5 that had been collected from Kano, Nigeria and 101-2-1 (Fig. 5.1I) that originated from Zambia.

It is clear from the aforementioned groupings that, the Bambara groundnut genotypes showed common relationships with individuals bearing distinct origins. It is therefore possible that the landraces may have common origins, which suggests that there may be frequent and free movement of seed materials from one region to another. The results showed that the Bambara groundnut landraces have sufficient genetic diversity for breeding purposes. Comparing the PC analysis and PCA biplot, the observed associations showed how the landraces share common certain traits. Similar observations were made by Shegro *et al*. (2013) who suggested the additional use of molecular markers to confirm such associations.

The value of PCA had been demonstrated by Ntundu *et al*. (2006) and Shegro *et al*. (2013) in order to predict associations of characters on Bambara groundnut accessions. In this study, major contributions to traits' association were displayed by PC_1 and PC_2 and were responsible for high Eigen values (Table 5.6). PC_1 was invariably responsible mostly for agronomic traits including leaf colour at emergence, petiole colour, leaflet joint pigmentation and calyx colour, while PC_2 was important for seed traits including seed length, seed width and seed height.

Table 5.5 Eigen values, proportion of variability and morphological traits that contributed to the nine PCs of Bambara groundnut genotypes

Traits	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9
Seed emergence (Days count)	-0.1	-0.267	0.112	0.201	-0.065	0.19	-0.425	0.498	0.386
Plant height (cm)	0.018	0.285	0.185	**0.814**	-0.143	-0.237	-0.15	-0.109	0.035
Canopy spread (cm)	0.211	0.141	0.475	0.475	-0.245	-0.002	0.217	-0.25	-0.057
Terminal leaf length (cm)	0.125	-0.013	-0.232	**0.895**	-0.049	0.244	0.088	-0.011	-0.022
Terminal leaf width (cm)	-0.179	0.413	0.192	-0.467	-0.193	-0.492	-0.149	-0.192	0.063
Petiole length (cm)	0.16	0.336	0.5	0.629	0.041	-0.11	-0.16	0.008	-0.029
Pod weight (gm)	-0.155	-0.051	**0.949**	-0.052	-0.066	0.03	-0.052	0.004	-0.021
Seed weight (gm)	0.359	0.329	-0.152	-0.18	0.168	-0.239	0.128	0.06	0.59
Biomass weight (gm)	-0.117	-0.064	**0.961**	0.044	-0.064	0.025	-0.041	-0.001	-0.067
100 seed weight (gm)	-0.083	0.152	-0.04	-0.172	-0.083	**0.768**	-0.09	-0.031	-0.245
Seed length (mm)	0.148	**0.77**	0.112	0.292	0.026	0.328	0.047	0.008	0.016
Seed width (mm)	0.062	**0.897**	-0.123	0.068	0.03	0.004	-0.003	0.06	0.178
Seed height (mm)	0.219	**0.892**	-0.016	0.059	-0.12	0.009	0.072	-0.071	-0.1
Leaf colour at emergence	**0.946**	0.097	-0.049	0.049	0.054	-0.089	-0.014	0.032	0.016
Leaf shape	0.134	0.076	0.01	-0.077	0.117	0.054	0.088	**0.83**	0.029
Growth habit	-0.008	-0.201	0.082	-0.048	-0.606	0.262	-0.061	-0.344	-0.163
Stem pigmentation	-0.291	-0.016	-0.104	-0.014	0.023	0.002	-0.224	0.003	**0.732**
Petiole colour	**0.949**	0.102	-0.105	0.126	0.024	-0.059	0.03	-0.026	-0.04
Leaflet joint pigmentation	**0.976**	0.097	-0.038	0.063	0.027	-0.042	-0.024	0.021	-0.052
Calyx colour	**0.976**	0.097	-0.038	0.063	0.027	-0.042	-0.024	0.021	-0.052
Fresh pod colour	0.071	-0.071	-0.054	0.036	**0.851**	-0.002	0.04	0.075	-0.193
Pod shape	-0.167	-0.072	0.315	-0.016	-0.309	0.038	0.503	0.3	-0.156
Pod colour	0.032	-0.12	-0.064	-0.266	**0.714**	0.031	-0.135	-0.063	0.197
Pod texture	0.004	0.062	-0.133	0.008	0.032	0.025	**0.909**	-0.029	-0.044
Seed shape	-0.204	0.073	0.087	0.114	-0.049	0.553	0.07	0.022	0.197
Seed eye pattern	-0.159	-0.158	-0.212	-0.17	0.12	-0.289	-0.103	0.484	-0.38
Eigen-values	5.125	3.736	2.408	2.098	2.031	1.614	1.377	1.205	1.034
Proportion variance (%)	19.711	14.369	9.261	8.069	7.81	6.207	5.297	4.636	3.976
Cumulative variance (%)	19.711	34.079	43.34	51.409	59.219	65.426	70.723	75.359	79.336

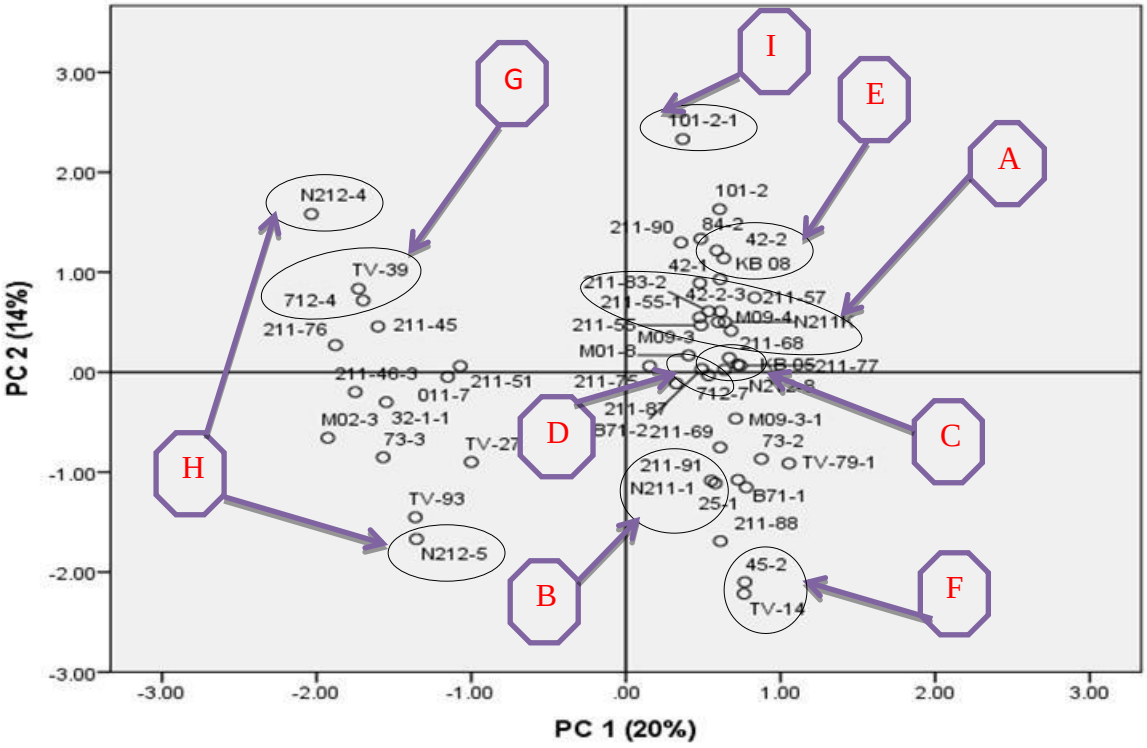

Fig. 5.1 Rotated principal component scores and percentage explaining variance of PC_1 versus PC_2 and showing similarities among 49 Bambara groundnut genotypes. Descriptions of the sources of the landraces used are indicated in Table 2.1.

5.6 Cluster analysis

The degree of relatedness and differences among 50 Bambara groundnut genotypes, which included all the 26 traits accessed in this study, are presented in a dendrogram (Fig. 5.2). The analyses displayed four major cluster groups that mostly comprise of heterogeneous genotype combinations. Cluster I consist of three genotypes including two from Zambia (712-4 and 45-2) bearing Tan seed coat colour, and one genotype (TV-93) from Sudan (acquired from IITA), which had a cream seed coat colour. The two Zambian genotypes were probably the same genotype, while the inclusion of the genotype from Sudan suggests that the three genotypes probably have a common origin or exhibit similarities in certain morphological features. The second cluster (Cluster II) was the largest, comprising of 24 genotypes distributed within two sub-clusters II a, and II b. Cluster II a, consisted of 19 genotypes, while II b had five genotypes. Cluster II a, is further divided into six sub-clusters II a1–II a6. The first sub-cluster, II a1, had an isolated genotype (TV-79-1) from IITA which originated from Kenya. The second, third, fourth and fifth sub-clusters (II a2, II a3, II a4 and II a5) consist of six genotypes, each embracing all seven geographical collection centers. Cluster II a6 had nine genotypes, with two forming a sub-sub-cluster (II a6-1) comprising of two genotypes, TV-14 and N212-4, from Ghana and Nigeria. Therefore TV-14 and N212-4 may have come from the same ancestral origin. The other sub-sub-cluster, II a6-2 included six genotypes out of which four (211-68,

211-51, 211-88 and 211-90) were from CAPS, while the remaining two, M09-3-1 and M12-1, both originated from Zimbabwe. Since CAPS manages and sells Bambara groundnut seeds comprising of mixtures of landraces, the inclusion of the last two genotypes from Zimbabwe suggest that the accessions in this cluster may have had the same origin.

Cluster II b and II b1 consisted of only one genotype each, TV-27 and N212-5, respectively. TV-27 was from IITA, and originated in Nigeria, while N212-5 originated from Kano in Nigeria, as well. Both genotypes did not associate with any genotypes in the Principal Component biplot (Fig. 5.1), suggesting that the two had unique origins in Nigeria, which were not similar between the two or with the other genotypes used in this study from the country. Furthermore, cluster II b1 formed two sub-clusters, II b1-1, which comprise of two sub-sub clusters, II b2-1 and II b2-2 (Fig. 2). Cluster II b2-1 was made up of two genotypes (101-2 and 101-2-1) from Zambia. The genotype 101-2-1 was a selection from 101-2, and the two had in common their seed coat colour (cream stripe) (Table 5.1). However, these two genotypes were not associated as indicated in the Principal Component biplot (Fig. 5.1). These relationships between Principal Component biplot and the cluster analyses suggest that there are certain inherent factors that made the two genotypes different. Such inherent factors could be understood further using molecular marker evaluation. The sub-cluster II b2-2 embraced eight genotypes, six of which originated from Zambia, whereas TV-39 and N212-8. TV-39 (IITA), originated from Sudan, and N212-8 was a collection from Kano in Nigeria. Although the genotypes in this sub-cluster (II b2-2) did not show any association in the Principal Component biplot, they still may have common or similar origin, or may share similar morphological attributes.

The clustering of the Bambara groundnut genotypes displayed to some extent homogeneity with the Principal Component biplot. For instance, the two genotypes 712-4 and TV-93, both of which appeared in Cluster I, had a close association (Fig. 5.1), but were distinct in seed coat colour (Tan and Dark brown), respectively (Table 5.1). Also, 45-2 and TV-14 were associated in the Principal Component biplot, but in a different quadrant than the previous genotypes. However, they also differed as well in seed coat colour; 45-2 was tan, while TV-14 was cream. This divergence, when Principal component biplot and cluster analysis are compared, means that the genotypes share common origin or similar traits among the 26 traits that were studied. Similarly, N211K, 211-55, M09-3 and 211-75 that were clustered in the sub-cluster II a5 showed a close association in the Principal Component biplot, and had cream seed coat colours except for 211-55 which was red. The Bambara groundnut genotypes did not cluster based on their geographical origin, but clustered according to a combination of agronomic and seed morphology, in addition to origin. Reports in this study are contrary to that of a morphological diversity of landraces in Tanzania by Ntundu *et al.* (2006), who observed that most of the landraces were grouped, according to their regional collection zones. Similarly cluster grouping based on collection location was reported in cowpea in Ghana (Cobbinah *et al.*, 2011). In this study, the clustering and grouping of the genotypes used suggested

that they may have a similar origin, in addition to sharing morphological attributes. The heterogenic nature with which the landrace collections exist would also allow for two or more landraces to have been the same seed material, but bearing different names, depending on where it was grown. Hence a concerted effort for further and advanced morphological and genomic characterization across Africa is important (Amadou *et al*., 2001).

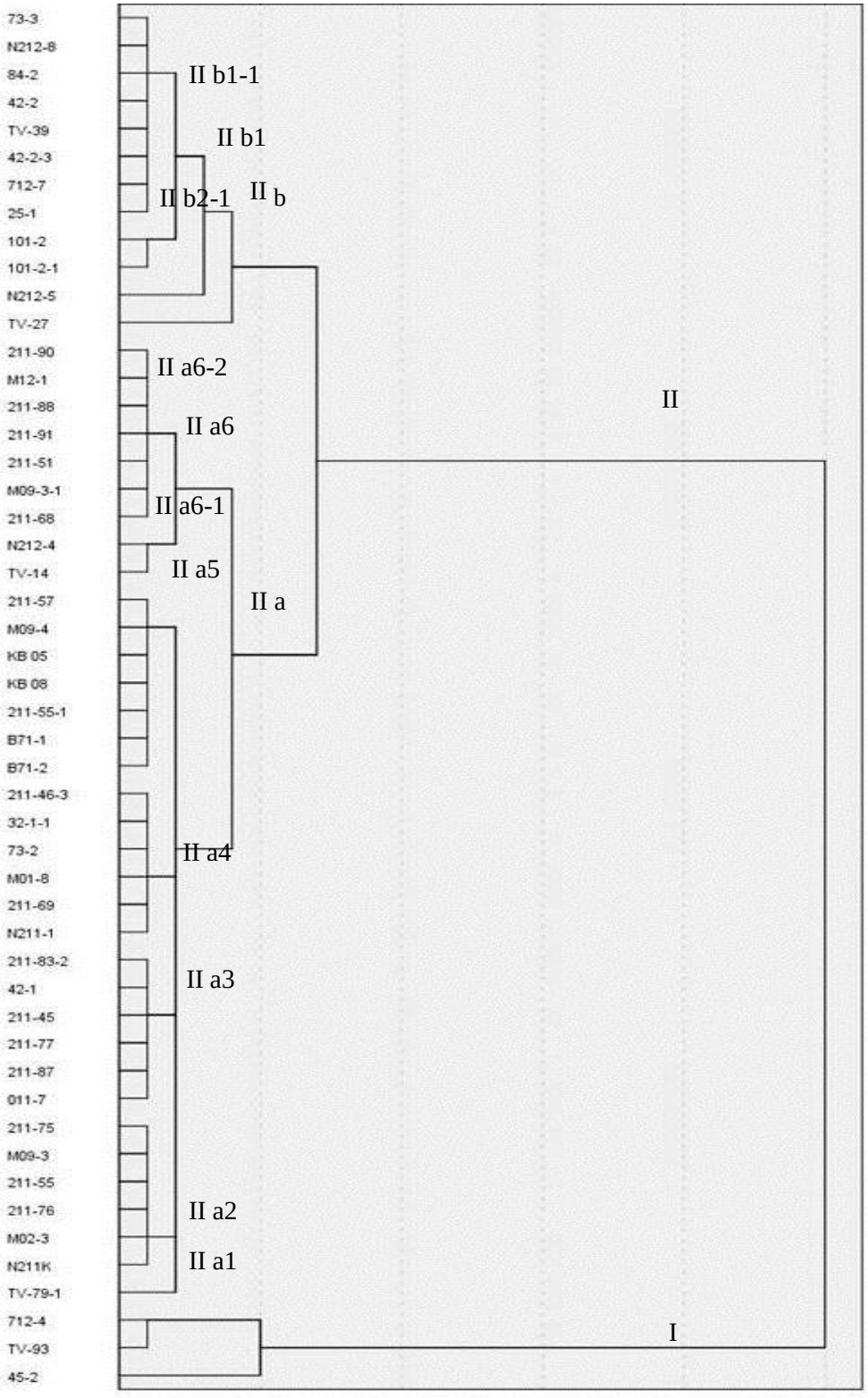

Fig. 5.2 Dendrogram based on average linkage for 13 quantitative and 13 qualitative characters of 49 Bambara groundnut genotypes. Description of the sources of the genotypes used are indicated in Table 1

5.7 Conclusion

Significant genetic variability has been reported for Bambara groundnut landraces (Masindeni, 2006; Ntundu *et al*., 2006; Shegro *et al*., 2013). Furthermore, it was observed that both Principal Component and cluster analyses did not purely group the landraces according to their origin, but according to morphological characteristics of the genotypes that were included. Bambara groundnut landraces were moved freely across the African sub-region during transportation and migration. As such, one landrace may have two or more identities depending on where it is collected. Use of biochemical and molecular markers may be an option to ascertain the genotype of any landraces collection prior to evaluating their agronomic worthiness; and to further enhance the speed of improvement of Bambara groundnut. This may also eliminate the use of similar landrace materials in different breeding programs with a similar aim of increasing food security in Africa.

In this study, three genotypes, 211-57, MO9-4 and TV-27 which originated from CAPS, Zimbabwe and Nigeria, respectively, had the highest seed yield and biomass production. There was also a relatively good association of the seed traits of seed length, seed width and seed height. On the other hand, genotypes TV-93 and 45-2, which originated from Kenya and Zambia respectively, showed a good performance in biomass production. These two genotypes would be important for fodder development. The best genotypes would be useful as breeding lines for cultivar improvement, large scale production or conservation.

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CHAPTER SIX

Genetic diversity of Bambara groundnut genotypes (*Vigna subterranea* [L.] Verdc.) revealed by SSR markers

Abstract

Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is an under-utilized legume crop of African origin which has substantial potential to contribute to food security in Africa. Limited research has been conducted on the genetic diversity, selection and breeding of the crop, especially using genomic tools. Bambara groundnut landraces have been previously characterized using morphological markers whose expression is heavily influenced by environmental factors. Molecular markers provide a better choice for genetic diversity studies because they are not affected by environmental factors or the growth stage of the crop species. Among them, SSRs have been found to be most convenient for genetic analysis with Bambara groundnut genotypes, especially because they are multiallelic, co-dominant and evenly dispersed throughout the genome. The objective of the study was to genotype 50 Bambara groundnut genotypes that were obtained from seven geographical regions across Africa, using five selected polymorphic simple sequence repeats (SSRs) markers developed for Bambara groundnut. The analyses detected a total of 53 alleles, with a mean of 10.6 alleles per locus, while genetic distance (D_A) as measured by polymorphic information content (PIC) ranged from 0.0 to 3.8, with a mean of 0.76. The neighbor-joining analysis generated seven major genetic groups, where the genotypes were clustered irrespective of their geographic origin. The study demonstrated the ability of the selected SSR markers to distinguish and group the Bambara groundnut genotypes which is useful for strategic breeding and genetic conservation of the crop.

Keywords: Bambara groundnut, genetic distance, microsatellite markers, neighbor-joining analysis, simple sequence repeats.

6.1 Introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc. $2n=2x=22$) is an African legume originating from West Africa (Hepper, 1963). The crop is primarily grown by resource poor farmers as a source of cheap protein (Massawe *et al*., 2005). Seeds of Bambara groundnut are consumed in fresh form as a vegetable, while in dry form the seeds are processed into flour to prepare other kind of foods as snacks (Linnemann and Azam-Ali, 1993). This makes Bambara groundnut a complement to cereal-based diet (Olukolu *et al*., 2012), hence this crop has the potential of reducing food insecurity in Africa (Shegro *et al*., 2013). Furthermore, the seeds are processed for animal feed, and leaves used as fodder (Ntundu *et al*., 2006). The crop is relatively drought tolerant and can grow where other

legumes may fail (Collinson *et al*., 1997) and shows some level of resistance to insect pests and diseases (Thottappilly and Rossel, 1997).

Bambara groundnut is a self-pollinating crop belonging to the family Leguminosae, sub-family Papilionoideae and genus *Vigna* (Fatokun *et al*., 1993). It is one of the most popular, but under-utilized grain legumes, with limited research interest by the scientific community (Amadou *et al*., 2001). Bambara groundnut landraces have been developed by farmers selecting and maintaining local varieties for production. Landraces may be distinguishable by their names, seed coat colour, growing locations, or markets (Massawe *et al.,* 2002). One landrace variety may bear several names due to the movement of seeds from one region to another. Presently, more than 2000 accessions have been collected and preserved by International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria (Massawe, 2000; Olukolu *et al*., 2012).

A major limitation to large scale production of Bambara groundnut in Africa is its low yield which is estimated to be as low as 68.5-159.9 kg ha^{-1} (Collinson *et al*., 2000). This has been attributed to lack of improved varieties (Mayes *et al*., 2008) and poor production technologies. Genetic enhancement of this valuable crop is essential to its productivity in the region. Genetic variation is the basis for Bambara groundnut breeding. Some genetic diversity studies have been reported on Bambara groundnut landraces, predominantly using morphological and agronomic traits (Ntundu *et al*., 2006; Olukolu *et al*., 2012).

Both morphological and molecular diversity analysis of Bambara groundnut can be used for genetic diversity analyses for subsequent breeding and release of varieties with desirable qualities including increased yield, and resistance to pests and diseases, abiotic stress tolerance and seed quality. Molecular markers have been used for gene mapping, mapping of quantitative traits loci (QTLs) and gene pyramiding for desirable traits such as agronomic, insect pest and disease resistance and stress tolerance, construction of linkage map and identification of polymorphism among segregating population (Collard and Mackill, 2008), as well as estimation of genetic diversity (Massawe *et al*., 2002).

Biochemical and molecular analyses of genetic diversity between and within Bambara groundnut landraces were reported. The most widely used were amplified fragment length polymorphism (AFLP) (Massawe *et al*., 2002; Ntundu *et al*., 2004), randomly amplified polymorphic DNA (RAPD) (Amadou *et al*., 2001, and SDS-polyacrylamide electrophoresis technique (Odeigah and Osanyinpeju, 1998).

RAPD, AFLP and SAPL (selectively amplified microsatellite polymorphic locus) have demonstrated some level of variability among cowpea (*Vigna unguiculata* [L.] Walpers) landraces (Tosti and Negri, 2002). The RAPD and AFLP markers showed high levels of polymorphism among Bambara groundnut landraces (Massawe *et al*., 2002; Singrun and Schenkel, 2004). RADPs identified

considerable polymorphism ranging from 63.2 to 88.2% with a mean of 73.1% among Bambara groundnut landraces at the Tropical Research Unit, University of Nottingham, UK (Massawe *et al*., 2003). RAPDs identified significant polymorphism among Bambara groundnut varieties grown in Namibia (Mukakalisa *et al*., 2013). Amadou *et al*. (2001) used RAPDs and investigated Bambara groundnut accessions from IITA, aligning their geographical origin into two groups.

Distinctive variation was found among Bambara groundnut accessions collected from different regions in Tanzania, and showed the ability of AFLP markers in assessing their diversity (Ntundu *et al*., 2004). Similarly, Fatokun *et al*. (1993) reported remarkable variation, using RFLP analyses among four legume subgenera, including soybean (*Glycine max* [L.] Merr), common bean (*Phaseolus vulgaris* L., mungbean (*Vigna radiata* L. Wilczek) and cowpea. Assessment of biodiversity among Bambara groundnut accessions have also been measured using SDS-polyacrylamide electrophoresis (Odeigah and Osanyinpeju, 1998). SSR markers have been used in diversity analysis of various legume crops such as in common bean (Blair *et al*., 2006).

SSR markers for diversity analysis have also been used in Bambara groundnut (Basu *et al*., 2007b; Tantasawat *et al*., 2010; Somta *et al*., 2011). A combination of restriction fragment length polymorphism (RFLP) markers, RADPs and SSRs were used to identify QTLs controlling seed weight in soybean (Maughan *et al*., 1996). Tantasawat *et al*. (2010) found high polymorphism in genetic diversity study using SSRs and ISSRs (inter-simple sequence repeats) among accessions of yardlong bean (*Vigna unguiculata* spp *sesquipedalis* L.).

The SSR markers also known as microsatellites have been found to be markers of choice for diversity studies. Being PCR-based, SSRs are technically simple to deploy and are amenable to high throughput assays (Mansfield *et al*., 1994), as well as being easy to score and requiring small amount of DNA for analysis (Somta *et al*., 2011). In recent years, the application of SSRs has been established in early generation selections among breeding populations (Gupta and Varshney, 2000).

Molecular markers offer greater power for detecting diversity that exceeds that of traditional methods (Gupta and Varshney, 2000). DNA markers including SSRs that are linked to agronomic traits could increase the efficiency of classical breeding by significantly reducing the number of backcross generations required and by reducing expensive, tedious, phenotypic selection as well as germplasm conservation. DNA markers also have the benefit that they can be used efficiently, regardless of the developmental stage of the plant under investigation (Mondini *et al*., 2009). There is scant information on the use of SSRs in Bambara groundnut genetic diversity studies. A recent study found SSRs to be the markers of choice for Bambara groundnut genetic diversity studies (Somta *et al*., 2011). Somta *et al*. (2011) employed SSRs markers tested on other legumes belonging to the Bambara groundnut genus', the '*Vigna* cultigens' including adzuki bean (*Vigna angularis* [Willd.]) and mungbean. These markers identified sufficient variability among the assessed Bambara groundnut

landraces. Bambara groundnut is a prominent member of the genus *Vigna;* hence its genetics may be similar or closely related to members of the same genus. SSRs markers were also employed by Basu *et al*. (2007a) to assess the genetic diversity of Bambara groundnut genotypes.

The objective of this study was to genotype 50 contrasting Bambara groundnut genotypes obtained from seven geographical regions across Africa using five selected polymorphic SSR markers developed for Bambara groundnut.

6.2 Materials and methods

6.2.1 Plant materials

Fifty Bambara groundnut genotypes from seven geographical locations were used for the study which originated (Table 6.1). All genotypes were pure breeding lines of single plants selected from earlier diversity study of within and between Bambara groundnut landraces. Selection of the accessions was based on distinct features of seed and plant morphological diversity.

6.2.2 DNA extraction and genotyping

Seeds were used for genomic DNA extraction. All samples were used in bulked amplification using DNA extracted from 7 coleoptiles per sample. A CTAB extraction procedure (CIMMYT, 2005) was followed. PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa).

Table 6.1 List of the Bambara groundnut genotypes and their origins used in the study

S/No.	Genotype	Origin	Seed coat colour	S/No.	Genotype	Origin	Seed coat colour
1	211-77	CAPS	Cream	26	211-75	CAPS	Cream
2	211-87	CAPS	Black	27	211-46-3	CAPS	Red
3	211-55	CAPS	Red	28	211-83-2	CAPS	Cream
4	32-1-1	ZM	Light brown	29	712-4	ZM	Tan
5	45-2	ZM	Tan	30	N211-1	KNG	Cream
6	211-55-1	CAPS	Red	31	KB 05	ARC	Cream
7	TV-79-1	IITA (Kenya)*	Cream	32	211-68	CAPS	Cream
8	211-90	CAPS	Black	33	101-2	ZM	Cream stripe
9	211-51	CAPS	Red	34	KB 08	ARC	Cream *RBF***
10	211-91	CAPS	Light brown	35	M12-1	ZIM	Cream
11	42-2-3	ZM	Light brown	36	712-7	ZM	Tan
12	84-2	ZM	Red	37	211-45	CAPS	Red
13	N211K	KNG	Cream	38	101-2-1	ZM	Cream stripe
14	73-3	ZM	Red	39	42-2	ZM	Light brown
15	211-76	CAPS	Cream	40	M01-8	ZIM	Cream *RBF*
16	25-1	ZM	Light brown	41	TV-93	IITA (Kenya)	Cream
17	B71-2	ARC	Cream	42	M02-3	ZIM	Cream *RBF*
18	M09-4	ZIM	Cream	43	B71-1	ARC	Cream
19	N212-5	KNG	Brown	44	73-2	ZM	Red
20	TV-27	IITA (Nigeria)	Dark brown speckle	45	211-88	CAPS	Black
21	M09-3-1	ZIM	Cream	46	N212-4	KNG	Brown
22	011-7	PMB	Cream stripe	47	TV-39	IITA (Sudan)	Dark brown speckle
23	N212-8	KNG	Brown	48	211-69	CAPS	Cream
24	211-57	CAPS	Red	49	M09-3	ZIM	Cream
25	42-1	ZM	Light brown	50	TV-14	IITA (Ghana)	Cream

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa; ***RBF***=Red butterfly eye

Five SSR markers (Table 6.2) specific for Bambara groundnut (Basu *et al*., 2007a; Somta *et al*., 2011) were used to perform the PCR reactions and analysis for genetic diversity among the Bambara groundnut genotypes.

The SSR primers used in this study were selected based on their high PIC and amplified alleles, and that they were developed being specific for Bambara groundnut (Basu *et al*., 2007a; Somta *et al*., 2011). Somta *et al*. (2011) compared PIC estimates among derived SSRs markers from three legumes including cowpea, adzuki bean and Bambara groundnut that revealed mean PIC estimates of 0.43, 0.61 and 0.78 for cowpea, adzuki bean and Bambara groundnut accessions, respectively. Means for allelic richness were 2.80, 2.90 and 3.75, respectively, for the same species. Among the Bambara groundnut SSRs markers used in this study, mBam2Co80 and mBam2Co33 had higher alleles score (8 and 12) per locus and PIC estimates (0.8 and 0.88) than seven others (Basu *et al*., 2007a). Sequences of the SSRs are presented in Table 6.2. An automated genetic analysis was employed to screen the SSR markers, using an automated gene sequencer (an ABI 3130 from Applied Biosystems, Johannesburg, South Africa). The analysis comprises the use of electrophoresis for amplification, wherein SSR loci that comprise of more than two base pairs may not be determined on agarose gel electrophoresis and nucleotides composed of up to 200bp (Sibov *et al*., 2003).

Table 6.2 Description of the SSRs markers used in this study

Marker name	Forward primer	Reverse primer	Source
mBamCo17	AACCTGAGAGAAGCGCGTAGAGAA	GGCTCCCTTCTAAGCAGCAGAACT	(Somta *et al.*, 2011)
mBam3Co39	CAGTAGCCATAATTTGCTATGAACA	CACATCAATCAAAAATCTCGGTAG	(Basu *et al.*, 2007b)
mBam2Co33	ATGTTCCTTCGTCCTTTTCTCAGC	AAAACAATCTCTGCCCCAAAAAGA	(Somta *et al.*, 2011)
mBam3Co07	GGGTTAGTGATAATAAATGGGTGTG	GTCATAGGAAAGGACCAGTTTCTC	(Somta *et al.*, 2011)
mBam2Co80	GAGTCCAATAACTGCTCCCGTTTG	ACGGCAAGCCCTAACTCTTCATTT	(Basu *et al.*, 2007b)

6.2.3 Data analysis

Analysis was performed using GeneMapper 4.1. The program GGT 2.0 (van Berloo, 2008) was used to calculate the Euclidian and Jaccard distances between bulked samples, and the matrix of the genetic distances was used to create a UPGMA and Neighbour Joining (NJ) dendrogram of the results.

6.3 Results and discussion

6.3.1 Marker characterization

The SSRs markers detected a total of 53 alleles with a mean of 10.6. A minimum number of six alleles were detected by the SSR marker, mBamC039, while mBam2C033 detected the most alleles which as 17 (Table 6.3). The mean alleles observed in this study was higher than 7.59 (Somta *et al*., 2011) and 5.20 (Basu *et al*., 2007) who also used the SSR markers employed in this study.

Table 6.3 Information of the SSR loci repeat type, bin location, number of alleles, PIC values and heterozygosity (He) for five SSR markers that were applied on fifty Bambara groundnut genotypes

SSR locus	Repeat type	No. of alleles	PIC value	He
mBam3C07	$(CT)_{22}$	9	0.7641	0.7940
mBamC017	$(GA)_{12}$	11	0.8486	0.8634
mBam2C033	$(CT)_{12}N_{47}(CT)_{16}(CA)_9$	17	0.8118	0.8322
mBam3C039	$(GT)_9(GA)_4$	6	0.5576	0.6261
mBam2C080	$(TG)_{17}(GA)_{13}$	10	0.7948	0.8170
Total		53	3.7769	3.9327
Mean		10.6	0.7554	0.7865

The polymorphic information content (PIC) describes the usefulness of SSR markers in identifying genetic similarities and differences among the pure lines, in this case, of the Bambara groundnut genotypes. It also, confirms the validity of using specific maker(s) in the construction of genetic linkage maps for the crop (Massawe *et al*., 2002). This maximizes selection of genetically distinct parents that can be used for the genetic enhancement of the crop (Amadou *et al*., 2001; Massawe *et al.*, 2002). The PIC observed in this study varied from 0.5576 to 0.8486, with a mean of 0.7554, as revealed by mBam3C039 and mBamC017 markers, respectively. A mean PIC of 0.58 was previously generated by 22 polymorphic SSRs markers in a diversity study among Bambara groundnut accessions from diverse origins (Somta *et al*., 2011) with range of 0.10 to 0.91 and a higher PIC of 0.70 which also revealed 166 alleles from the same materials. Use of SSRs on other legumes include mungbean (PIC=7.3) and blackgram (PIC=4.1) (Danzmann *et al*., 2009).

The allelic diversity, as explained by heterozygosity (*He*), varied between 0.6261 and 0.8634 for mBam3C039 and mBamC017 markers, respectively. This range is higher than the scores of 0.54 and 0.77 reported for the same markers by Basu *et al*. (2007a). Somta *et al*. (2011) reported the highest mean PIC and *He* of 0.70 and 0.552, respectively. Bambara groundnut being self-fertilizing, the findings in this study compared favourably with previous reports, because, the genotypes used were from single plant selection which were pure lines. As such it is probable that the selected plants used for analysis in the previous study were from heterogeneous mixtures of landrace seeds. Somta *et al*. (2013) employed a cross-species amplification of SSRs on 34 Bambara groundnut accessions which detected between 2 and 8 alleles per marker, and a PIC estimate of 0.16 to 0.73, while none of the markers revealed any heterozygosity among the accessions. This underlines the detection power of the markers that were used in this study for effective genetic grouping of the 50 Bambara groundnut genotypes. The SSR markers which were developed for Bambara groundnut (Basu *et al*., 2007a), have generally revealed high correlations between the PIC and *He* estimates. They also match with the allelic detection by the corresponding markers, with mBamC017 and mBamCo33 markers presenting higher correlation between

PIC values of 0.8486 and 0.8118, and *He* values 0.8634 and 0.8322, respectively. These means were higher than those reported by Basu *et al*. (2007a) and Somta *et al*. (2011) using SSRs including those used in this study. High PIC estimates describe the strength of the molecular markers, especially SSRs that have the advantage of being co-dominant and multiallelic (Gupta *et al*., 2003), to distinguish any variability among species, which is resolved by the number and frequency of alleles discovered (Somta *et al*., 2011). The results explained the homogenous status of the genotypes used in this study as sourced from single plant selection, i.e. pure lines. The findings in this study suggest that these SSR markers could be used in any Bambara groundnut genetic diversity study and genetic map construction.

6.3.2 Genetic distance

The genetic distance (D_A) among the 50 Bambara groundnut genotypes from the seven geographical locations are presented in Table 6.4, with a minimum of 0.0 to a maximum D_A 3.8 among 11 pairs of genotypes. This difference in the D_A of (0.00 to 3.8) observed in this study is lower than the values 0.28 and 0.27 and 0.53 and 0.53 the minimum and the maximum distances among Bambara groundnut landraces from two extreme geographical locations of Togo (Africa) and Thailand (Asia) (Somta *et al*., 2011). The extent of variation among the landraces used in the previous study was higher than that observed in the current genetic analysis. The findings in the current study revealed that the Bambara groundnut genotype 211-68 from CAPS (South Africa) correlated at a D_A of 0.0 each with 211-83-2 also from CAPS, as well as N211K and M09-3, which originated from Kano in Nigeria and Zimbabwe, respectively. N211K had a close association with two genotypes, 211-51 and 211-83-2, which originated from CAPS. These correlations link genotypes from the two distinct geographical locations, Kano in Nigeria and CAPS in South Africa which suggested that the genotypes involved may have a common origin. In addition, the genotypes 101-2 and 101-2-1 from Zambia displayed similar relationship with D_A at 0.0. M12-1 from Zimbabwe is related to 211-91 from CAPS, and 211-57 and 211-55-1 suggests similar origin. TV-93 and TV-79-1 have a close association.

The distance of 0.30 on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram (Fig. 6.1) between M12-1 and 211-91, and that between TV-93 and TV-79-1, reflected the extreme similarity between the two pairs, suggesting that these two pairs may be the same genotypes. This D_A of 0.0 emphasizes the capacity of the SSR markers to discriminate among the Bambara groundnut genotypes, even between those that have close relationships. Similarly, it was observed that most of these genotypes, including M09-3, 211-68, 211-51 and 211-83-2, were grouped in the same cluster on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram (Fig. 6.1). Furthermore, close and similar associations with a D_A of 3.6 were detected between KB 05 from ARC in South Africa and 211-551and 211-57 from CAPS and KB 08 from the ARC in South Africa and 211-55-1 and 211-57 from CAPS. These relationships may be explained by the fact

that CAPS is a seed company that sells Bambara groundnut landraces composed of seed mixtures. It is based in South Africa as is the ARC. We propose that the genotypes have common origins. Interestingly, KB 05 and KB 08 on one hand, and 211-55-1 and 211-57 were grouped on the same, but separate 'leaves' (simplicifolious) on the Jaccard Neighbor-joining (Jaccard NJ) dendrogram in the II and III clusters, respectively. Hence, this result also showed the ability of the SSR markers to distinguish between genotypes that are distinct, similar or closely related. In their genetic diversity study using RAPD Massawe *et al*. (2003) found a similar trend of association, and proposed that such close associations between Bambara groundnut landraces could mean that they were related or that they were the same genotypes. Similar suggestions were made by Ntundu *et al*. (2006) in a morphological diversity study among Bambara groundnut landraces in Tanzania. These authors proposed that unorganized collection and grouping of Bambara groundnut landraces would result in a single genotype bearing several names (Massawe *et al*., 2003).

The highest D_A of 3.8 was observed between two pairs of Bambara groundnut genotypes, M02-3 and 211-51-1, and M02-3 and 211-57 (Table 6.4). However, these two pairs were not grouped in the same cluster (Fig. 6.1). Amadou *et al*. (2001) used RAPD markers and found that Bambara groundnut accessions from Zambia and Zimbabwe were grouped in the same cluster, suggesting that the same seed material may have been taken from one of the location to the other. The D_A observed in this study revealed low minimum and maximum values, when compared with reports of other genetic studies based on SSRs (Somta *et al*., 2011), AFLP (Ntundu *et al*., 2004) and RAPD (Amadou *et al*., 2001; Massawe *et al*., 2003). These variations may be due to the of nature the germplasm used in this study, which consisted of pure lines from single plant selection, compared to the use of landraces composed of mixtures of a few to several seed morpho-types.

Table 6.4 Similarity matrix based on Euclidean NJ coefficient for the 50 Bambara groundnut genotypes used in the study

Genotypes	011-7	25-1	32-1-1	42-1	42-2	42-2-3	45-2	73-2	73-3	84-2	101-2-1	101-2	211-45	211-46-3	211-51	211-55-1
25-1	3.6															
32-1-1	2.5	2.2														
42-1	2.0	2.4	2.4													
42-2	1.9	2.1	2.3	0.5												
42-2-3	2.3	2.1	1.0	1.9	1.8											
45-2	1.6	3.5	2.1	2.0	2.2	1.9										
73-2	2.5	2.7	2.9	1.0	1.3	2.1	2.2									
73-3	1.5	3.6	2.4	1.9	2.1	1.8	1.1	2.1								
84-2	1.6	2.5	1.2	1.9	1.7	1.2	1.9	2.5	1.8							
101-2-1	2.3	2.7	1.2	2.1	2.2	0.7	1.6	2.3	1.5	1.4						
101-2	2.3	2.7	1.2	2.1	2.2	0.7	1.6	2.3	1.5	1.4	0.0					
211-45	1.5	3.7	2.6	1.9	2.1	2.5	1.1	2.5	1.7	2.1	2.3	2.3				
211-46-3	1.5	3.6	2.6	1.7	1.9	2.3	1.5	2.3	1.4	1.8	2.1	2.1	1.0			
211-51	2.1	2.8	1.9	1.9	1.9	1.5	2.3	2.5	1.7	1.1	1.5	1.5	2.4	1.7		
211-55-1	3.1	2.3	1.6	2.8	2.6	1.6	3.2	3.3	2.9	1.6	1.9	1.9	3.5	3.0	1.5	
211-55	2.3	2.8	2.2	2.4	2.1	2.5	2.9	3.4	3.0	1.5	2.7	2.7	2.4	2.2	2.0	2.3
211-57	3.1	2.3	1.6	2.8	2.6	1.6	3.2	3.3	2.9	1.6	1.9	1.9	3.5	3.0	1.5	0.0
211-68	1.5	2.6	1.3	1.7	1.6	1.1	1.5	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1.0	1.8
211-69	1.4	3.2	2.3	1.7	1.7	2.1	2.1	2.5	1.8	1.2	2.1	2.1	1.8	1.1	1.1	2.3
211-75	1.4	3.2	2.3	1.7	1.7	2.1	2.1	2.5	1.8	1.2	2.1	2.1	1.8	1.1	1.1	2.3
211-76	1.6	2.9	1.6	1.6	1.7	1.2	1.2	2.1	1.1	1.0	1.0	1.0	1.5	1.1	1.1	2.1
211-77	3.0	2.3	1.2	2.4	2.4	1.2	2.5	2.9	2.5	1.6	1.2	1.2	2.9	2.5	1.5	1.2
211-83-2	1.5	2.6	1.3	1.7	1.6	1.1	1.5	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1.0	1.8
211-87	2.3	2.8	1.7	2.8	2.5	1.5	2.5	3.0	2.2	1.5	1.8	1.8	3.2	3.0	2.0	1.8

Table 6.4 Continued

Genotypes	211-55	211-57	211-68	211-69	211-75	211-76	211-77	211-83-2	Genotypes	011-7	25-1	32-1-1	42-1	42-2	42-2-3	45-2
011-7									211-88	2.5	1.7	2.4	0.9	0.7	1.8	2.5
25-1									211-90	2.1	2.5	1.0	2.5	2.3	1.6	2.1
32-1-1									211-91	1.1	3.2	2.2	1.7	1.6	2.1	1.8
42-1									712-4	2.3	1.9	2.2	0.9	0.7	1.5	2.4
42-2									712-7	2.1	2.5	2.8	0.9	0.7	2.3	2.6
42-2-3									B71-1	2.1	2.1	1.4	1.6	1.5	1.2	1.6
45-2									B71-2	1.9	2.7	1.0	2.1	2.1	1.2	1.2
73-2									KB05	3.0	2.5	3.0	1.3	1.6	2.6	2.6
73-3									KB08	2.5	2.7	3.0	0.9	1.2	2.4	2.2
84-2									N211-1	2.5	3.2	2.2	2.6	2.5	1.5	2.5
101-2-1									N211K	1.4	2.6	1.2	1.7	1.6	1.1	1.1
101-2									N212-4	2.3	3.6	2.6	2.3	2.5	1.9	2.1
211-45									N212-5	3.1	1.8	1.9	2	1.9	1.2	2.8
211-46-3									N212-8	2.1	3.7	2.8	2.2	2.3	2.1	2.1
211-51									M01-8	1.6	2.7	1.7	2.1	1.8	1.9	2.3
211-55-1									M02-3	1.9	3.0	3.0	1.2	1.5	2.6	1.8
211-55									M09-3-1	1.1	3.3	1.9	2.2	2.1	1.8	1.1
211-57	2.3								M09-3	1.5	2.6	1.3	1.7	1.6	1.1	1.5
211-68	1.7	1.8							M09-4	2.5	3.0	3.3	0.9	1.2	2.7	2.5
211-69	1.5	2.3	1.1						M12-1	1.1	3.2	2.2	1.7	1.6	2.1	1.8
211-75	1.5	2.3	1.1	0.0					TV-14	1.9	2.5	1	1.9	1.8	0.7	1.6
211-76	2.1	2.1	0.5	1.2	1.2				TV-27	2.1	2.7	1.2	2.2	2.2	0.7	1.7
211-77	2.3	1.2	1.5	2.2	2.2	1.6			TV-39	1.2	2.9	1.6	1.7	1.7	1.2	1.4
211-83-2	1.7	1.8	0.0	1.1	1.1	0.5	1.5		TV-79-1	1.2	3	1.9	2.1	1.9	1.6	1.9
211-87	2.8	1.8	1.7	2.5	2.5	2.1	2.3	1.7	TV-93	1.2	3	1.9	2.1	1.9	1.6	1.9

Table 6.4 Continued

Genotypes	73-2	73-3	84-2	101-2-1	101-2	211-45	211-46-3	211-51	211-55-1	211-55	211-57	211-68	211-69	211-75	211-76	211-77
211-88	1.1	2.4	2.1	2.3	2.3	2.6	2.4	2.2	2.7	2.6	2.7	2.0	2.3	2.3	2.1	2.5
211-90	3.1	2.5	1.2	1.9	1.9	2.5	2.7	2.3	2.1	2.1	2.1	1.5	2.3	2.3	1.9	2.1
211-91	2.5	1.7	1.1	2.1	2.1	1.4	1.0	1.4	2.5	1.4	2.5	1.0	0.5	0.5	1.1	2.3
712-4	1.3	2.1	1.7	1.9	1.9	2.5	2.1	1.6	2.2	2.3	2.2	1.6	1.8	1.8	1.7	2.1
712-7	1.7	2.3	1.9	2.6	2.6	2.3	1.9	1.9	2.8	2.1	2.8	1.9	1.5	1.5	1.9	2.7
B71-1	1.9	2.1	1.6	1.6	1.6	2.1	2.3	2.3	2.5	2.5	2.5	1.5	2.3	2.3	1.6	2.1
B71-2	2.5	1.8	1.2	1.2	1.2	1.8	2.1	2.1	2.3	2.3	2.3	1.1	2.1	2.1	1.2	1.9
KB05	1.7	2.9	2.8	2.8	2.8	2.3	2.3	2.9	3.6	2.9	3.6	2.5	2.7	2.7	2.4	2.9
KB08	0.9	2.3	2.6	2.6	2.6	2.1	2.1	2.7	3.6	3.1	3.6	2.3	2.5	2.5	2.2	3.0
N211-1	2.7	1.7	1.8	1.5	1.5	3.2	2.6	1.4	1.8	3.2	1.8	1.7	2.3	2.3	1.8	2.1
N211K	2.3	1.4	0.5	1.1	1.1	1.4	1.4	0.0	1.5	1.7	1.5	0.0	1.0	1.0	0.5	1.4
N212-4	2.3	1.1	2.1	1.6	1.6	2.7	2.1	1.5	2.5	3.4	2.5	1.8	2.1	2.1	1.6	2.3
N212-5	2.2	2.5	1.9	1.6	1.6	3.2	2.7	1.5	1.4	2.7	1.4	1.8	2.3	2.3	1.9	1.2
N212-8	2.3	1.0	2.1	1.8	1.8	2.4	1.7	1.4	2.7	3.2	2.7	1.7	1.8	1.8	1.5	2.5
M01-8	2.9	2.3	0.7	2.1	2.1	2.3	2.1	1.5	1.9	1.1	1.9	1.1	1.2	1.2	1.6	2.1
M02-3	1.5	2.1	2.5	2.7	2.7	1.5	1.8	2.9	3.8	2.7	3.8	2.3	2.3	2.3	2.1	3.1
M09-3-1	2.5	1.4	1.5	1.8	1.8	1.7	2.0	2.2	2.9	2.6	2.9	1.4	2.1	2.1	1.5	2.7
M09-3	2.3	1.4	0.5	1.1	1.1	1.7	1.4	1.0	1.8	1.7	1.8	0.0	1.1	1.1	0.5	1.5
M09-4	1.1	2.4	2.7	2.9	2.9	2.2	2.0	2.6	3.6	3.0	3.6	2.4	2.3	2.3	2.3	3.2
M12-1	2.5	1.7	1.1	2.1	2.1	1.4	1.0	1.4	2.5	1.4	2.5	1.0	0.5	0.5	1.1	2.3
TV-14	2.3	1.5	0.7	0.7	0.7	2.1	1.8	1.1	1.6	2.1	1.6	0.5	1.6	1.6	0.7	1.2
TV-27	2.4	1.5	1.2	0.7	0.7	2.5	2.3	1.5	1.7	2.7	1.7	1.1	2.1	2.1	1.2	1.6
TV-39	2.2	1.1	0.7	1.2	1.2	1.8	1.5	1.1	2.0	2.1	2.0	0.5	1.2	1.2	0.7	1.9
TV-79-1	2.5	1.5	1.0	1.7	1.7	2.3	2.1	1.5	2.1	2.3	2.1	1.1	1.6	1.6	1.4	2.3
TV-93	2.5	1.5	1.0	1.7	1.7	2.3	2.1	1.5	2.1	2.3	2.1	1.1	1.6	1.6	1.4	2.3

Table 6.4 Continued

Genotypes	211-83-2	211-87	211-88	211-90	211-91	712-4	712-7	B71-1	B71-2	KB05	KB08	N211-1	N211K	N212-4	N212-5	N212-8
211-88	2.0	2.6														
211-90	1.5	1.5	2.5													
211-91	1.0	2.4	2.2	2.1												
712-4	1.6	2.3	0.7	2.4	1.9											
712-7	1.9	2.9	1.2	2.8	1.6	1.0										
B71-1	1.5	2.1	1.5	1.4	2.1	1.7	2.2									
B71-2	1.1	1.8	2.3	1.0	1.8	2.2	2.6	1.0								
KB05	2.5	3.8	1.6	3.2	2.5	1.9	1.9	2.1	2.7							
KB08	2.3	3.4	1.2	3.0	2.3	1.6	1.6	1.8	2.5	1.0						
N211-1	1.7	1.4	2.6	2.5	2.4	2.1	2.7	2.5	2.3	3.7	3.2					
N211K	0.0	1.7	2.0	1.1	1.0	1.5	1.8	1.1	0.5	2.5	2.3	1.4				
N212-4	1.8	2.3	2.7	3.0	2.3	2.2	2.6	2.6	2.4	3.4	2.9	1.1	1.5			
N212-5	1.8	2.3	1.8	2.5	2.5	1.3	2.2	2.1	2.3	2.6	2.6	1.8	1.5	2.1		
N212-8	1.7	2.4	2.6	3.0	2.0	2.1	2.3	2.7	2.5	3.2	2.7	1.4	1.4	0.5	2.3	
M01-8	1.1	1.8	2.3	1.4	1.1	1.9	1.9	2.0	1.7	3.0	2.9	2.3	1.1	2.6	2.3	2.5
M02-3	2.3	3.4	1.8	2.9	2.1	2.1	1.8	1.9	2.3	0.9	0.9	3.4	2.1	2.9	3.0	2.7
M09-3-1	1.4	1.7	2.4	1.5	1.7	2.3	2.5	1.5	1.1	3.1	2.5	2.2	1.0	2.3	2.9	2.2
M09-3	0.0	1.7	2.0	1.5	1.0	1.6	1.9	1.5	1.1	2.5	2.3	1.7	0.0	1.8	1.8	1.7
M09-4	2.4	3.6	1.4	3.4	2.2	1.6	1.2	2.3	2.9	1.2	0.7	3.3	2.4	2.9	2.7	2.6
M12-1	1.0	2.4	2.2	2.1	0.0	1.9	1.6	2.1	1.8	2.5	2.3	2.4	1.0	2.3	2.5	2.0
TV-14	0.5	1.5	2.1	1.4	1.5	1.7	2.2	1.4	1.0	2.7	2.5	1.5	0.5	1.7	1.6	1.8
TV-27	1.1	1.1	2.3	1.6	2.1	1.9	2.6	1.6	1.2	3.1	2.8	1.1	1.1	1.6	1.7	1.8
TV-39	0.5	1.5	2.1	1.6	1.1	1.7	1.9	1.6	1.2	2.8	2.4	1.5	0.5	1.6	2.0	1.5
TV-79-1	1.1	1.1	2.3	1.6	1.5	1.9	2.2	1.9	1.6	3.3	2.8	1.5	1.1	1.9	2.3	1.8
TV-93	1.1	1.1	2.3	1.6	1.5	1.9	2.2	1.9	1.6	3.3	2.8	1.5	1.1	1.9	2.3	1.8

Table 6.4 Continued

Genotypes	M01-8	M02-3	M09-3-1	M09-3	M09-4	M12-1	TV-14	TV-27	TV-39
M02-3	2.7								
M09-3-1	1.8	2.1							
M09-3	1.1	2.3	1.4						
M09-4	2.9	1.1	2.8	2.4					
M12-1	1.1	2.1	1.7	1.0	2.2				
TV-14	1.4	2.5	1.5	0.5	2.7	1.5			
TV-27	1.9	2.9	1.5	1.1	3.0	2.1	0.7		
TV-39	1.2	2.3	1.1	0.5	2.5	1.1	0.7	1.0	
TV-79-1	1.2	2.5	1.1	1.1	2.9	1.5	1.2	1.2	0.7

6.3.3 Genetic relationship

The levels of similarities and divergence among the fifty Bambara groundnut genotypes are presented in Fig. 6.1 and Table 6.4 using the Jaccard Neighbor-joining analysis. The analyses revealed the presence of significant genetic diversity among the tested genotypes. The genotypes were conveniently grouped into seven definite clusters, independent of geographical origin (Table 6.4). Conversely, Amadou *et al*. (2001) and Ntundu *et al*. (2004) collectively reported genomic grouping of Bambara groundnut landraces that were related to geographical origin using RAPDs and AFLP, respectively. The findings in this study demonstrated the ability of SSR markers to portion the genotypes into closer genetic groupings than other marker systems. The pattern was similar to that obtained in a morphological diversity study presented in the previous chapter.

The largest among the seven clusters was Cluster III which consisted of 12 genotypes emanating from four geographical sources (Fig. 6.1). Five of these genotypes originate from CAPS, three from Zambia, two from Kano and one from IITA (Table 6.5). Two genotypes, 101-2 and 101-2-1, were positioned closely in this cluster, with the latter being a selection from the former, suggesting that they possess similar genes. Cluster I followed with ten genotypes, of which six originated from CAPS, while three were sourced from Zimbabwe, and one genotype was obtained from a farmers' collection in Pietermaritzburg that appeared as an outlier.

Capstone Seed Company is a seed company in South Africa that buys and sells Bambara groundnut seeds composed of mixtures of different morpho-types. The seed lots vary in seed coat colour and eye pattern. Hence there is the possibility that CAPS may have secured Bambara groundnut seed landraces from Zimbabwe and other neighboring countries hence the grouping pattern.

Cluster II comprised of nine genotypes collectively originating from CAPS, Zambia and ARC in South Africa. In this cluster, two pairs of genotypes KB 05 and KB 08 from ARC in South Africa, and 42-1 and 42-2 from Zambia, had strong similarities. However, the two pairs varied in seed coat colour: while 42-1

was light brown, 42-2-2 was cream. The smallest cluster was Cluster IV which had only three genotypes, M01-8, which originated from Zimbabwe, while N211K and TV-14 originated from Kano and Ghana, respectively, reflecting a close genetic relationship, despite their distant origins.

Pasquet *et al*. (1999) compared the genetic diversity between wild and domesticated Bambara groundnut accessions using isozyme markers and reported a close relationship between the two species suggesting that the former is the progenitor of the latter. However, Ntundu *et al*. (2004) discussed isozymes as having limited use for genetic analysis due to their low levels of polymorphism.

The findings in this study confirmed the detection power of the SSRs to resolve the genetic diversity of the Bambara groundnut genotypes into their similarity and divergent groups with great precision, while each genotype was derived from single plant selection that was presumed to be genetically uniform.

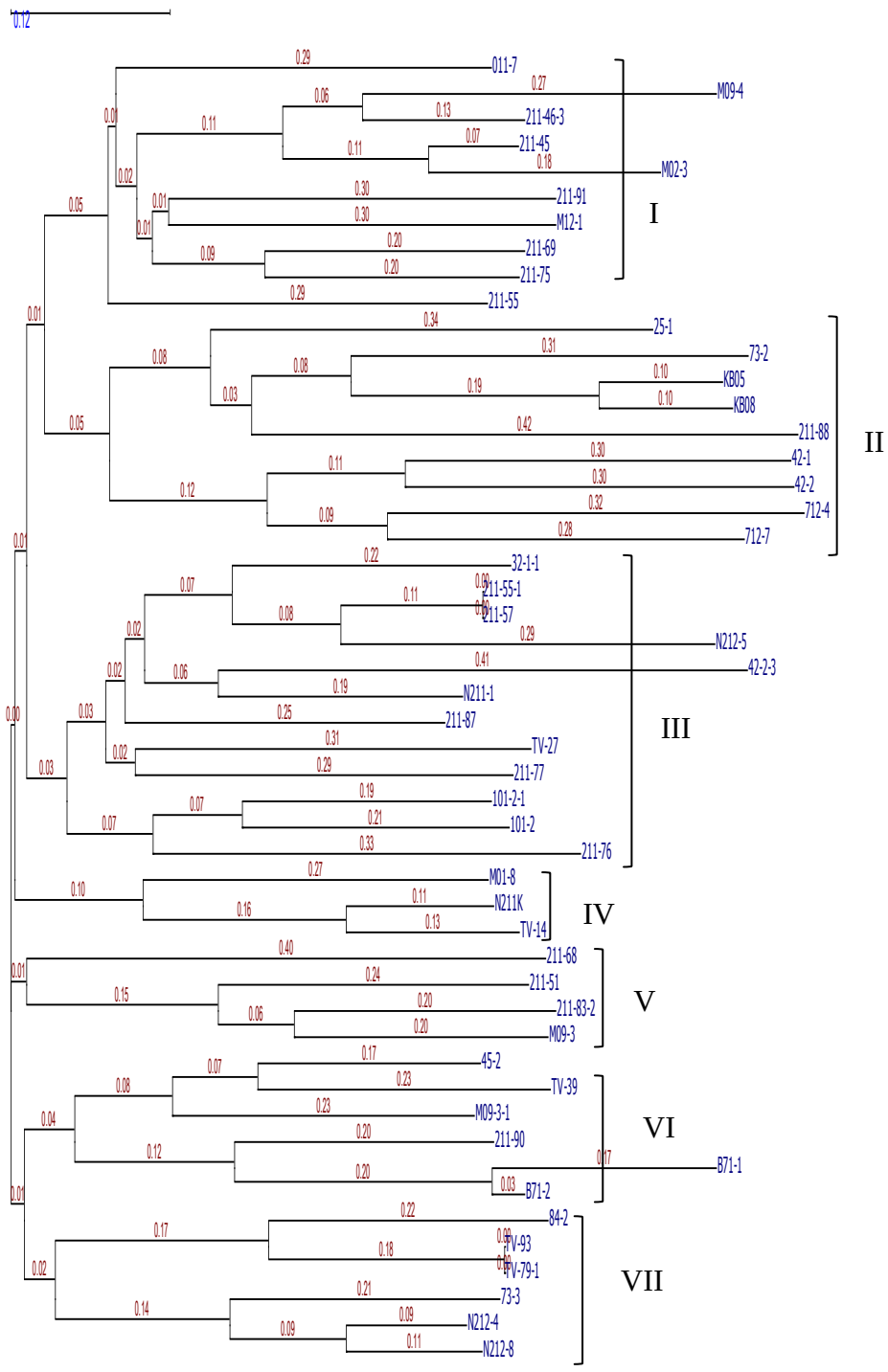

Fig. 6.1 The Jaccard Neighbor-joining dendrogram illustrating genetic diversity and relationships among 50 Bambara groundnut genotypes used in the study

Table 6.5 Cluster grouping of the fifty Bambara groundnut genotypes and their origin

Cluster	Genotype	Origin
Cluster I	211-46-3, 211-45, 211-91, 211-69, 211-75, 211-55	CAPS, South Africa
	M09-4, M02-3, M12-1	National Program, Zimbabwe
	011-7	PMB farmer collection
Cluster II	211-88	CAPS, South Africa
	25-1, 73-2, 42-1, 42-2, 712-1, 712-7	National Program, Zambia
	KB 05, KB 08	ARC, South Africa
Cluster III	211-51-1, 211-57, 21187, 211-77, 211-76	CAPS, South Africa
	32-1-1, 42-2-3, 101-2-1, 101-2	Zambia National Program
	TV-27	IITA, Ibadan Nigeria
	N212-5, N211-1	Kano farmers' collection
Cluster IV	M01-8	National Program, Zimbabwe
	TV-14	IITA
	N211K	Farmers' collection from Kano
Cluster V	211-68, 21151, 211-83-2	CAPS, South Africa
	M09-3	National Program, Zimbabwe
Cluster VI	211-90	CAPS, South Africa
	45-2	National Program, Zambia
	M09-3-1	National Program, Zimbabwe
	TV-39	IITA, Ibadan Nigeria
	B71-1, B71-2	ARC, South Africa
Cluster VII	84-2,73-3	National Program, Zambia
	TV-93, TV-79-1	IITA, Ibadan Nigeria
	N21-4, 212-8	Farmers' collection form Kano

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZAM** =The National Plant Genetic Resources Centre, Zambia; **ARC** =Agricultural Research Council, Republic of South Africa; **PMB** =Farmer collection from Pietermaritzburg in South Africa; **KNG** =Farmers' collection from Kano, Nigeria; **IITA** =International Institute of Tropical Agriculture, Ibadan in Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

6.4 Conclusion

The genetic analysis using the SSR makers revealed the extent of similarity and differences among the 50 Bambara groundnut genotypes used in this study, which compared favourably with the results obtained in similar studies (Basu *et al*., 2007b; Somta *et al*., 2011) using SSRs including those adopted in this study. In this study, PIC estimates varied from 0.5576 to 0.8486 with a mean of 0.7554, while heterozygosity (*He*) varied between 0.6261 and 0.8634 with a mean of 0.7865. These measurements were higher than the ranges of 0.70 and 0.552, and 0.54 and 0.77 of PIC and *He* found by Basu *et al*. (2007b) and Somta *et al*. (2011), respectively. In a different trial using a cross-species of SSRs Somta *et al*. (2013) found a range of 2 and 8 alleles per locus, and a PIC estimate of 0.16 to 0.73, while none of the markers revealed any heterozygosity among the accessions. There were also fewer alleles than those revealed in this study, 6 to 17 per locus with a mean of 10.6. High PIC estimates reflect the strength of the DNA markers, especially SSRs, having the advantage of co-

dominance and multiallelic to distinguish any differences among species, and to determine the number and frequency of alleles. Furthermore, the SSR analysis exhibited a comparable pattern between morphological diversity of the same genotypes (presented in Chapter Five of this study) and the result displayed in the Jaccard Neighbor-joining analysis. The outcome of the genetic distance analysis showed that the Bambara groundnut genotypes were grouped into seven clusters, consisting of combination of genotypes from different geographical origin. This was in contradiction of reports by (Ntundu *et al*. 2004; Somta *et al*. 2011) who respectively, used AFLP and SSRs markers and described grouping of Bambara groundnut landraces according to their geographical location or collection centers. Amadou *et al*. (2001) found grouping of Bambara groundnut landraces from two countries in the same cluster which is similar to what was observed in this study, suggesting that the indiscriminate transfer of landraces from one region to another, bearing different names and identities, but possessing the same genetic information. In addition, certain pairs of genotypes including 101-2 and 101-2-1 from Zambia both with cream stripe seed coat colour, and TV-93 and TV-79-1 sourced from IITA, originally from Kenya and both with cream seed coat colour, have a high proximity with one another, suggesting that they may be the same genotypes (Tables 6.1 and 6.4, and Fig. 6.1). However, the close affinity between KB 05 KB 08 (bearing cream and cream *RBF*, respectively) suggest that they were genetically close. Furthermore, KB 05 and KB 08 were observed to individually and equally associate closely with 211-55-1 and 211-57, suggesting possession of similar genes as well. Singular associations were found between 211-68 on one hand and 211-83-2, N211K and M09-3, indicating the possibility of their having common origin. Similar associations were recorded between M02-3, 211-55-1 and 211-57. These complex associations suggest the possibility that the genotypes involved may be the same, possessing similar genes or have common origins.

This study confirmed that the homogeneity of the genotypes used in this study was because they were sourced from single plant selections, i.e. pure lines. Bambara groundnut is self pollinating and strictly cleistagamous, whose flower opens after pollination occured. The SSR markers were highly effective at discriminating between the 50 Bambara groundnut genotypes.

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CHAPTER SEVEN

Preliminary investigation of the crossing of Bambara groundnut (*Vigna subterranea* [L.] Verdc.)

Abstract

Effective crosses among selected parents are crucial for genetic analyses and for the breeding of crop plants. Bambara groundnut is an indigenous African legume with considerable genetic diversity, which is useful when breeding for enhanced yield and quality traits. However, the crop has previously received limited research attention. This may be attributed to its extremely small flower size, its flower orientation, the delicate nature of the flower and its mating system. The aim of this study was to establish a preliminary crossing protocol for Bambara groundnut for breeding and genetic studies. Controlled emasculation and pollination were performed using eight selected parents, using a diallel mating scheme under glasshouse conditions. Some successful crosses were achieved and F_1 seeds were recovered from the crosses of 211-40-1 x N211-2, N212-8 x 211-40-1 and M09-3 x 211-82-1.

Keywords: Bambara groundnut, emasculation, crossing, pollination, F_1 hybrids

7.1 Introduction

Bambara groundnut is one of the most valuable grain legumes, native to Africa, which shares similar agro-ecology and growing environments with cowpea (Basu *et al.*, 2007). Bambara groundnut is a member of the Papilionaceae (Leguminosae) family, sub-family Papilionoideae (Fabaceae), genus *Vigna* and species *subterranea* (Fatokun *et al*., 1993). The species has two botanical varieties or sub-species: var. *spontanea* (the wild form) and var. *subterranea* (the cultivated form). Both are diploids with the chromosome number of 2n=22 (Frahm-Leliveld, 1953; Forni-Martins, 1986). The wild forms were found in 1909 in north-east of Nigeria, which supports the theory that the crop originated in West Africa (Dalziel, 1937). The crop spread to Asia and Latin America, probably through the slave trade, and is found in Sri-Lanka, Malaysia, Philippines and India, and Brazil (Rassel, 1960; Goli *et al*., 1997).

Bambara groundnut is an important source of dietary protein in sub-Saharan Africa, with protein levels of 16-25% (Brough *et al.*, 1993); carbohydrates and oil content is in the region of 55-72% and 6-7%, respectively (Suwanprasert *et al*., 2006). Fresh pods and seeds are eaten as a vegetable after boiling, like green peas. Dry seeds are roasted and eaten as a nutritionally balanced snack, while ground dry seeds are used to prepare many form of dishes such as Moi-moi, which is made from a steamed paste (Okpuzor *et al*., 2009). Bambara groundnut seed can be processed to make bread (Fetuga *et al*., 1975) and into vegetable milk similar to that made from soybean (Brough *et al.*, 1993). The paste can be fried in oil and be served as snack with porridge at breakfast. Bambara groundnut is

a source of balanced food, and makes an important contribution to food security, and to reducing protein malnutrition in rural communities in Africa (Ouedraogo *et al.*, 2008). The crop combines the advantage of drought tolerance and some high level of resistance to insect pests and diseases (Obagwu, 2003). Bambara groundnut is versatile and can produce a moderate harvest in environments where other legumes such as groundnut fail to produce a crop (Linnemann and Azam-Ali, 1993). And as a legume, Bambara groundnut possesses the ability to fix atmospheric nitrogen through the activity of the symbiotic bacteria (*Bradyrhizobium* species) in root nodules.

Bambara groundnut shows wide genetic variation and is predominantly grown as landrace varieties, consisting of mixed seeds that display several morpho-types. The International Institute of Tropical Agriculture (IITA) based in Ibadan, Nigeria has the mandate for Bambara groundnut research and germplasm conservation. The Institute has collected and preserved over 2,000 accessions whose genetic diversity has not been adequately characterized to select for further genetic improvement in any breeding program (Massawe *et al*., 2005). However, several research reports (Ofori, 1996; Goli *et al*., 1997; Ntundu *et al*., 2006; Onwubiko *et al*., 2011) indicated that some of the Bambara groundnut landraces had been characterized for their morphological attributes. The reports noted that there was enough genetic variation to conduct strategic breeding (Massawe *et al*., 2005).

Bambara groundnut is strictly a self-pollinating crop, bearing a perfect flower that stands on a short raceme attached to a long peduncle by the pedicle, alternately on stem nodes. The stamen, which is diadelphous, consists of 10 filaments that connect to the anthers on the tip carrying the pollen grains. The filaments are united into two sets: nine out of ten have their filaments fused, with one isolated vexillary stamen (Goli *et al*., 1997; Basu *et al*., 2007). The stigma becomes receptive earlier and the anthers dehisce shortly before the flowers opens. The pollen grains of Bambara groundnut are trinucleate and short lived after anthesis. The flowers are cleistogamous, (i.e. the flowers are tightly enclosed by petals and sepals, and open only after pollination), and therefore pollination occurs immediately after the anthers dehisce. Fertilization takes place on the day of anthesis and after pollination (Linnemann, 1992).

Uguru *et al*. (2002) used cytogenetic analyses to understand the genetics of the floral system that can be employed to successfully cross Bambara groundnut. However, research reports indicated the difficulty of genetic analyses and breeding of Bambara groundnut using conventional manual crosses (Goli *et al*., 1997; Suwanprasert *et al*., 2006; Koné *et al*., 2007). During conventional breeding, controlled emasculation and pollination of flowers are essential to recover progenies for targeted selection. Factors hindering the emasculation and crossing procedures of Bambara groundnut are: its small flower size, its flower orientation, the delicate nature of the flower and its mating system (Myers, 1991). Despite the difficulties associated with crossing of the Bambara groundnut, efforts have been made to undertake controlled crosses, and segregating populations have been generated (Massawe *et al*., 2004; Suwanprasert *et al*., 2006; Basu *et al*., 2007). Management of the unavoidable

variation in time-to-anthesis of different parental lines is critical for successful crossing, as reported by Suwanprasert *et al.* (2006) and Onwubiko *et al.* (2011). In addition, Oyiga and Uguru (2011) recommended the use of indole-3 acetic acid to enhance pollen germination. Suwanprasert *et al.* (2006) reported that the ideal emasculation time is between 3:00pm and 10:00pm, with successful crosses being made between 2:30 to 3:00am the next day. Onwubiko *et al*. (2011) suggested that pollination should be completed within 12 hours of emasculation, and that the blooming period ensues between 7:00am and 10:00am when pollination can be conducted. At the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), emasculation for crossing of groundnut, a related legume, is routinely carried out the between 1:30pm and 04:30pm, and pollination is conducted the following day between 6:00am and 08:00am (Nigam *et al*., 1990). In the case of cowpea Myers (1991) recommended that emasculation should be carried out in the evening between 4.00pm to 6.00pm, followed by pollination at 6.00am and 08.00am the next day when anthesis commences. These extreme differences in timing may be associated with the different environmental conditions under which the crosses were made, as well as genotypic and species differences.

Patel *et al*. (1935) showed that flowers in groundnut are blocked by bracts that make it difficult to get rid of unwanted flowers, which may result to selfing.

A detailed, simple, step-by-step protocol is not available for making crosses in Bambara groundnut for effective genetic analyses and breeding. In the light of this limitation, the aim of this study was to establish a preliminary crossing protocol for Bambara groundnut for breeding and genetic studies.

7.2 Materials and method

7.2.1 Selection of parents, planting and mating scheme

7.2.1.1 Selection of parents

Currently, seeds for Bambara groundnut production are available in the form of landraces, in which seed and plant morphology vary considerably. The present study used eight genotypes for the full diallel crosses (Table 7.1). The parents were kept true to type after rigorous selection with regards to source, uniform seed coat colour, and uniform seed eye and hilum patterns.

Table 7.1 Some of the seed characteristics of the Bambara groundnut genotypes used for the full diallel crosses

Name of genotype	ID number	Source	Seed coat colour	Eye pattern	Hilum colour	Seed size
ZIM 109-3	M 09-3	ZIM	Red	Plain	White	Medium
KN 211-2	N 211-2	KNG	Cream	Light-grey	White	Medium
PSC 211-51	211-51	CAPS	Black	Plain	White	Medium
ZM 6608-2	608-2	ZM	Brown	Plain	White	Medium
ZM 5712-3	712-3	ZM	White-cream	Plain	Chalk-white	Small
PSC 211-40-1	211-40-1	CAPS	Dark-brown	Plain	White	Small
KN 211-8	N 211-8	KNG	Cream-brown stripe	Light-brown thin	White	Medium
PSC 211-82-1	211-82-1	CAPS	Dark-brown black spots	Plain	White	Small

Legend on seed sources: ZIM =Department of Research and Specialist Services, Zimbabwe; **ZM** =The National Plant Genetic Resources Centre, Zambia; **KN** =Farmers' collection from Kano, Nigeria; **CAPS** =Capstone Seed Company, Howick, South Africa

7.2.1.2 Planting

To facilitate crossing, 32 plastic pots of 5 litre capacity, filled with a composted pine bark medium, were assigned to each of the eight parents into which two seeds were planted. Out of the 32 pots allocated for each of the eight genotypes, four were designated as male parents, while 28 were designated as maternal parents. The seed was planted on the 7th of January, 2013 in the glasshouse kept under controlled temperatures and humidity. The day and night temperatures of the glasshouse were 25^{0}C and 18^{0}C, respectively, while relative humidity was kept at 70 to 80%. Within one week after germination, the seedlings were thinned to one plant per pot to allow sufficient growth, development and ease of accessing the flowers during crossing. Pots with growing plants were placed on tables high enough for convenience for crossing (Fig. 7.1F).

7.2.1.3 Mating scheme

Crosses were established following an 8 x 8 full-diallel mating scheme. Each parent was grown in at least four plastic pots. Planting of these pots was staggered at an interval of 10 days to ensure synchronized flowering among parents, and to allow for effective crosses. Depending on the accessions, flowering begins from 35 days after planting. Before starting the emasculation and pollination procedures, the first few flowers were removed for about three days; this was to encourage sequential flower production from both pollen and maternal parents.

7.2.2 Emasculation

Blooming of the Bambara groundnut flowers occurred for a brief period, about 1 to 2 hours before sunrise, depending on the temporal changes in the summer months between November and March. Usually, flowers destined to open the next day on the maternal parent(s) were prepared for the emasculation (the removal of filaments with immature anthers before self-pollination) and pollination (the transfer of pollen grains from a male parent onto the stigma of a female parent). At this stage the colour of the flower bud changes from green to pale yellow, during which time the stigma is

receptive, but the anthers have not matured yet, and cannot deliver effective pollination and fertilization. On each day emasculation needs to begin between 4:30am and 5:00am and pollination needs to follow, between 8:30am and 9:00am.

This approach is contrary to the procedures reported by Onwubiko *et al*., (2011) and Suwanprasert *et al*. (2006). However, we found that it was more convenient to conduct both the emasculation and pollination steps on the same day. With our approach, a flower that is ready for emasculation is handled gently with the left hand using the thumb and the index finger. Using a pair of sharp scissors in the right hand a gentle cut is made, large enough to expose the stamens carrying the immature pollens, which is a cut of about 1/2 to 2/3 of the width of the unopened flower (Fig. 7.1A). Maximum care was taken to avoid damaging the flower in the process, because of the delicate nature of the flower bud. A cut was made from the side where the flower would be destined to open because the dorsal side contains the stamens. A pair of tweezers was used to gently pull out the cut the sepal and petal that enclose the stamen and pistil (Fig. 7.1B). The single and nine fused stamens were then shaved gently using tweezers, making sure that the stigma remains intact and undamaged. With care, the corolla, the standard and the stamens were removed at the same time. At this point the stigma is exposed and is ready for pollination.

A jeweler's loupe was used both during emasculation and pollination, in order to clearly see the small flower parts, and to ensure successful emasculation and pollination. To avoid contamination, 70% alcohol was used to clean both hands and all the tools used in making crosses at every step of the emasculation and pollination procedures between any two parents.

7.2.3 Pollination

Pollinations were carried out immediately after emasculation. The opening of flower buds begins at sun-rise, particularly on bright days. Pollination begins as the flowers open, typically from 5:30am until 9:00am. For pollination, a freshly opened flower was removed from the male parent as a source of pollen grains to be transferred to the stigma of the maternal parent. The anther sac was opened by tearing off the floral leaves (calyx, corolla and the wing). The anthers containing the pollen grains are squeezed out and placed onto the stigma of the maternal parent using a pollen brush. It was observed that flower size and the prevailing environmental condition affected pollen abundance. Therefore, at times up to 5 to 10 female flower were pollinated using one male flower. The keel top of the male flower was used to cap the stigma gently, to ensure pollen contact with the stigma.

Flowers that reach an advanced growth stage on the maternal parents but have not been hand-pollinated, and which are destined to open the next day were removed to avoid the development of any selfed seeds on maternal plants. The process also encouraged production of more flowers for future crosses. This activity was also practiced on the pollen parents, here to promote production of more flowers for use in forthcoming pollinations. Due to the small size of the Bambara groundnut

flowers, pollinated flowers were covered to avoid uncontrolled pollinations. Each emasculated and pollinated flower was tagged and labelled by tying a string of thread at each node for proper identification of developing pod and for effective monitoring (Fig. 7.2 C).

7.2.4 Cross confirmation and management of hybrids

On completion of the crosses, maternal parents were routinely checked to remove any developing flower bud to exclude selfed seeds. This exercise continued for four weeks. Fertilized flowers (Figs. 7.1 and 7.2), were monitored until the pods were matured and harvested. During this period an insect problem was encountered specifically that black ants (*Monomorium minimum*) damaged some of the crossed flowers and developing pods on the maternal parents. An insecticide (cypermethrin) was sprayed to eliminate the problem. The F_1 pods were harvested and dried, put in separate envelops and labelled according to crosses.

Fig. 7.1 Processes of emasculation and pollination of Bambara groundnut: (A) cutting a flower bud; (B and C) removing the anthers of the flower bud; (D and E) introducing pollen grains from the paternal parent to the stigma of the maternal parent; (F) conducting cross-pollination in a glasshouse

Fig. 7.2 Monitoring of Bambara groundnut F1 hybrids: (A) pegs of developing pods of the F_1 hybrid seed, towards the tip of the peg; (B and C) showing well developed Bambara groundnut F_1 pods

Note: Remains of the dried feathery stigma are shown using the arrows on the developing pods (A), and on the well-developed pods (C), suggesting that the pods are derived from crosses, although this can only be confirmed when the F_1 seeds are phenotyped or genotyped.

7.3 Results and discussion

Results of the attempted crosses are presented in Table 7.2. M09-3, 211-1 and N211-2 were good parents, providing 62 pods. The cross M09-3 x 211-40-1 generated 8 F_1 pods from 32 crosses, while the cross 211-40-1 x N211-2 generated 8 F_1 pods from 14 crosses, which was the highest number of F_1s among the entire 8x8 diallel. Overall, 21 F_1s were produced using N212-8 as the male parent, when crossing onto the other seven parents as females, followed by 17 F_1s when 211-51 was used as the male parent. The crossing technique described above was successful, although the numbers of hybrid seed generated were not sufficient for genetic analyses at the F_1 generation. However, the F_1 seed can be selfed and genetic analyses can be conducted on the F_2 or even the F_3 generations.

Success of crossing in the common groundnut has been shown to be influenced by the mishandling of flower buds by breeders or technicians, the prevailing environmental conditions and the genotypes involved (Nigam *et al*., 1990). In this study all the genotypes were selections based on uniform seed

morphology. There was no any prior information available on their agronomic attributes and nature of flowering.

Cross-pollination of Bambara groundnut can be achieved by way of simultaneous emasculation and pollination on the same day, between 4:30am and 9:00am. F_1 hybrids were obtained from each of the cross combinations in the 8 x 8 diallel. In Thailand, Suwanprasert *et al*. (2006) carried out pollination of Bambara groundnut earlier in the morning at 2:30am and 3:30am but this may reflect the environmental differences between Thailand and the South Africa

Despite the flower size being smaller than those of cowpea and groundnut, Bambara groundnut can be improved through conventional crossing techniques.

Table 7.2 Number of successful crosses and F_1 pods harvested from 8 x 8 diallel crosses of Bambara groundnut

MALE	712-3		M09-3		N212-8		N211-2		608-2		211-51		211-40-1		211-82-1	
FEMALE	Successful Crosses	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods	Successful Cross	F_1 Pods
712-3	**X**	**X**	12	0	14	0	9	1	14	2	21	2	21	3	2	0
M09-3	7	0	**X**	**X**	10	0	7	4	6	2	5	0	14	0	8	5
N212-8	9	0	12	2	**X**	**X**	4	0	9	1	12	5	32	8	7	3
608-2	20	3	7	2	12	1	12	5	**X**	**X**	9	3	0	0	3	2
211-51	8	1	8	0	9	0	6	0	7	0	**X**	**X**	8	2	7	1
211-40-1	9	3	12	0	12	0	14	8	12	5	12	6	**X**	**X**	14	1
211-82-1	4	0	10	0	5	0	16	3	13	2	7	1	0	0	**X**	**X**
N211-2	5	0	0	0	7	0	**X**	**X**	2	0	0	0	0	0	4	0
TOTAL	**62**	**7**	**61**	**4**	**69**	**1**	**68**	**21**	**63**	**12**	**66**	**17**	**75**	**13**	**45**	**12**

Key: Cells within columns marked '**X**' are selfs

7.4 Conclusion

In this study, a protocol for the cross-pollination of Bambara groundnut was developed, despite small flower size of the crop which makes this process difficult. A key development was that the pollination step was conducted immediately after the emasculation step, which is contrary to protocols used to make crosses in cowpea and groundnut. In these protocols, emasculations are done the previous day and pollinations follow the next day. The protocol developed here will help breeders of Bambara groundnut to make crosses for genetic analyses and for breeding for the genetic enhancement of the crop. Relative to reports on the success of other crossing procedures used on groundnut and cowpea, and the crossing techniques used on Bambara groundnut previously, the improved protocol used here produced more F_1 seeds within the limited blooming period of Bambara groundnut, because both emasculation and pollination were carried out one after the other. Furthermore, the protocol could reduce the extent of flower damage from the interval between emasculation and pollination employed on groundnut, cowpea and Bambara groundnut, as reported by Nigam *et al*. (1990), Myers (1991) and Suwanprasert *et al*. (2006), respectively.

The limitation of this study was that few F_1 seeds were produced because of the difficult nature of crossing the Bambara groundnut flowers. Hence there is there is a need for more crosses using the same genotypes to obtain sufficient number of F_1 seeds that can be used for genetic analyses on the F_2 or F_3 generations.

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Thesis Overview

Introduction and objectives of the study

Bambara groundnut (*Vigna subterranea* [L.]Verdc.; **Syn:** *Voandzeia subterranea* [L.] Thouars.) is an under-utilized grain legume grown in Africa, mostly cultivated by women for food security (Ntundu *et al.*, 2006). It is the most important legume crop in Africa after groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* [L.] Walp.) (Sellschop, 1962; Linnemann and Azam-Ali, 1993). The seed of Bambara groundnut contains approximately 20% protein, 63% carbohydrates and 18% oil, and the fatty acid content is predominantly oleic, palmitic and linolenic acids (Minka and Bruneteau, 2000). This nutritional profile makes it a good complement for cereal-based diets in Africa. Bambara groundnut has the potential to improve nutrition, boost food security, foster rural development and support sustainable land use. Despite the varied socio-economic importance, the crop has a low research and development status in Africa.

In the region, Bambara groundnut germplasm has been maintained as landraces, which are often phenotypically and genetically diverse. The Bambara groundnut landraces represent local varieties that have evolved largely through random natural crosses, followed by selection by farmers. These landraces are well adapted to the prevailing agro-ecologies, and to produce crops despite limited agronomic inputs such as fertilizers. The landraces can be systematically exploited in **breeding** programs after a systematic **pre-breeding programme,** which requires the application of a set of procedures designed to identify desirable characteristics and/or heritable genes from un-adapted and unimproved plant genetic materials and their subsequent manipulation in the actual breeding programmes (Nass and Paterniani, 2000). Pre-breeding is a vital step that links conservation and use of plant genetic resources with formal plant breeding, which leads to the genetic enhancement of the crop for desirable agronomic characteristics. Procedures of pre-breeding include the development of new parent populations to be used as breeding materials, with the long-term goal of using the best parents for cultivar development following progeny testing; introgression of new traits from other useful sources, usually a landrace or related species; and the creation of novel traits through the use of various plant breeding techniques such as mutation breeding. Therefore, the main focus of this study was to initiate a dedicated Bambara groundnut pre-breeding programme as the first step of systematically breeding this valuable crop to create improved cultivars.

This overview compares the original study objectives with the major research findings relative to each objective. Finally, the implications of the findings are presented in terms of their contributions to the future of Bambara groundnut breeding.

Objectives

Specific objectives of this study were initially established as follows:

To assess the production status and constraints associated with the farming of Bambara groundnut in the Kano State of Nigeria;

To determine the diversity of seed morphology of Bambara groundnut germplasm collections from seven different sources across Africa;

To determine the inter-and intra-morphological diversity of Bambara groundnut landraces collected from seven different sources;

To evaluate selected pure line Bambara groundnut landraces for yield and important yield component traits;

To determine the genetic diversity of selected Bambara groundnut genotypes using SSR markers;

To optimize a protocol for the crossing of Bambara groundnut; by employing the protocol, a diallel cross will be performed to determine the levels of heterosis, and general and specific combining abilities for a set of qualitative and quantitative characters, to be found in a selection of Bambara groundnut accessions.

Research findings in brief

Assessment of the production status and constraints associated with Bambara groundnut in the Kano State of Nigeria

A baseline survey, using Participatory Rural Appraisal (PRA) was carried out among seven Local Government Areas (LGAs) in Kano State, Northern Nigeria to study the production status, farming practice, production constraints and farmers' variety preferences of Bambara groundnut. During this survey, 27 diverse landraces bearing different names were identified in the hands of the farmers. Of these, the most popular were Gurjiya, Kurasa, Hawayen-Zaki, Fara Mai-Bargo and Silva. The most important production constraints among the Bambara groundnut farmers were lack of improved varieties, frequent drought, low yield and limited access to market, while preferred attributes of improved varieties were oval and large pure seeds with cream seed coat colour and early maturing. This emphasized that the improvement of improving Bambara groundnut should be centered on these characters.

Determination of the diversity of seed morphology of Bambara groundnut germplasm collections from seven different sources across Africa

Bambara groundnut is an under-utilized grain legume whose seed commonly exist as landraces in popular growing regions across Africa (Ntundu *et al.*, 2006). The result of the PRA involved the identification of Bambara groundnut farmers' production constraints and preferred traits in an improved variety, provided the basis for the acquisition of Bambara groundnut landraces from seven diverse geographic origins. These landraces were characterized using seed morphology including seed

coat, seed eye colour and pattern, and hilum colour and pattern to identify novel genotypes for breeding. From a total of 58 collections, a further 353 different seed morpho-types were identified; these can further be used for large-scale production or true-to-type lines that could be used in genetic improvement of the crop.

Determination of the inter-and intra-morphological diversity of Bambara groundnut landraces collected from seven different sources

A set of Bambara groundnut seeds were selected from the previous study for whose genetic variability within- and between-landraces was investigated among 262 landraces, where 49 were studied for agronomic traits, and 213 were investigated for pod and seed variability. Another set of 158 landraces were evaluated for their leaf morphology, out of which 49.4% had round leaves, while 21.5% had elliptic leaves, and 55.7% landraces were morphologically heterogeneous, possessing more than one form of leaf shapes. The result revealed wide variability among pod, seed and leaf morphology that can be exploited through single plant selection that can be used as breeding lines, as well as their use in breeding and selection of desirable genotypes bearing improved characters.

Characterization and evaluation of selected pure line Bambara groundnut landraces for yield and important yield component traits

Single plant selection of 49 Bambara groundnut genotypes was made from the genetic variability of within- and between-landraces carried out earlier. These genotypes, which represented collections from seven geographical regions across Africa, were characterized and evaluated for yield and yield related traits. They showed high variability for canopy spread, petiole length, weight of biomass, seed weight and seed height. Principal component analysis (PCA) identified nine influential components from wherein two components, PC_1 and PC_2, contributed immensely to the total variation, at 19% and 14%, respectively. Among the selected genotypes, 211-57, MO9-4 and TV-27 produced the highest seed yields, while the genotypes TV-93 and 45-2 produced the higher total biomass. The PCA facilitates identification of unique characters that can be used for identification of hybrids during hybridization and selection. Therefore, genotypes possessing yield related characters and those associated with PCA will provide breeding lines that can be used for the Bambara groundnut enhancement and conservation.

Determination of the genetic diversity of selected Bambara groundnut genotypes using single sequence repeat (SSR) markers

Fifty Bambara groundnut genotypes which included those evaluated for yield and yield components were genotyped using five pre-selected polymorphic simple sequence repeats (SSRs) markers, previously developed by others for Bambara groundnut (Basu *et al*., 2007). The SSR analysis revealed a total of 53 alleles and the genotypes were clustered, irrespective of their geographic origin, suggesting the possibility the genotypes were spread across the collection regions and/had common

origins. The result indicated the ability of the SSR markers to show the genetic status of the Bambara groundnut genotypes used in the study. These SSR markers can be useful in a marker assisted breeding for Bambara groundnut.

Optimization of a protocol for crossing Bambara groundnut, and performance of diallel crosses to determine heterosis and general and specific combining abilities of qualitative and quantitative characters among selected Bambara groundnut genotypes

Attempts of unsuccessful crosses were reported of Bambara groundnut at different times of the day at the International Institute of Tropical Agriculture (Goli, 1997). Similar crossing failures were reported by Schenkel (2000) and Massawe *et al*. (2003). Previously, it was discovered that both pollen maturity and stigma receptivity of the flower ensue just before or immediately the flower opened (Doku and Karikari, 1971). In addition, Oyiga *et al*. (2010) opined that shedding of pollen and artificial hybridization should not last >5 minutes. In the light of this a preliminary crossing protocol for Bambara groundnut was designed, where controlled cross pollinations (emasculation and pollination) were carried out among eight selected parents, using an 8x8 diallel mating design for breeding and genetic studies. Emasculation and crossing of the Bambara groundnut were achieved on the same day, with both exercises conducted sequentially, in the morning between 04:30 am to 09:00 am**.** The protocol was successful, but yielded a limited number of F_1 seeds, with the most successful crosses being between 211-40-1 x N211-2, N212-8 x 211-40-1 and M09-3 x 211-82-1. However, the number of generated F_1 seeds would not be sufficient for genetic analysis, suggesting the need for repeated crosses or the advancement of these F_1 seeds to confirm true crosses and growing the latter to produce F_2 or F_3 populations for use in the genetic analysis.

Overall, the study generated valuable and novel Bambara groundnut genetic material useful in the development of improved cultivars for large-scale production in sub-Saharan Africa. Genotypes that excelled in seed yield and biomass can be used as breeding lines for genetic improvement of the crop. The crossing protocol designed in this study provides a fast and simple procedure that can be employed to speed the generation of segregating populations for selection and release of improved Bambara groundnut varieties to growing regions.

Future Research

Genotypes possessing unique characteristics that comply with the farmers' preferred attributes of improved varieties, were identified through the PRA study. These can be utilized for the development of new varieties that satisfy the farmers' need, especially using the Bambara groundnut crossing protocol presented in this thesis. SSR markers earlier developed for Bambara groundnut were successfully applied in this study which can be employed to screen segregating population to identify breeding lines possessing desirable traits in a marker-assisted breeding for the crop. This will ensure

the speedy release of improved varieties of Bambara groundnut to the growers. Furthermore, the diverse features of the seed morpho-types and important plant characteristics identified from the seed morphological characterization and PCA analysis, respectively, can also be employed for systematic genetic analysis of Bambara groundnut. This may eventually be useful for varietal development and genetic conservation.

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APPENDIX I
A Copy of the Questionnaire Used for the Participatory Rural Appraisal Conducted in Kano State Nigeria

M.S. Mohammed is a Postgraduate Student in the School of Agricultural, Earth and Environmental Sciences, College of Agriculture, Engineering and Sciences in the discipline of Plant Breeding of the University of KwaZulu-Natal of the Republic of South Africa. Mohammed is conducting a survey on the production and production constraints associated with Bambara groundnut in Kano State, Nigeria. Information made available will be kept confidential, but will be used towards Mohammed's PhD Thesis in the discipline of Plant Breeding. Your cooperation is greatly appreciated.

The enumerator

Name of enumerator	
Title	
Zone	
Local Government Area	
Block	
Village (Cell)	

Socio-demography of respondent

Name of respondent	
Name of village/township (Cell)	
Age	
Gender (Male or Female)	
Marital status (Married or Single)	
Level of education (Last attended)	

Farming history and production practice of the respondent

For how long have you been a farmer?

- <2 years
- 2-5 years
- 5-7 years
- 7-10 years
- >10 years

What is/are your source(s) of agricultural extension services?

- Government extension personnel
- Non-governmental organizations
- Mass media
- Agricultural retailers
- Neighboring farmers
- Others

What inputs do you acquire for your farm production?

Production inputs	**Priority (1-6 scale)**
Seeds	
Fertilizer	
Herbicides	
Insecticides	
Fungicides	
Storage materials (e.g. sacks, etc)	
Storage chemical	
Others	

What is/are the source (s) of your farm inputs?

- Government agencies □
- Local leaders □
- Non-governmental organizations □
- Agricultural retailers □
- Neighboring farmers □
- Others □

What farming practice(s) do you engage?

- Sole cropping □
- Mixed cropping □
- Mixed farming □
- Subsistence □
- Large scale □

Production of Bambara groundnut and other legumes crops compared

What types of crops do you produce, the acreage and harvest?

Legumes	**Acreage**	**Harvest (Kg ha^{-1})**
Bambara G/nut		
Cowpea		
Groundnut		
Soybeans		
Others		
Cereals	**Acreage**	**Harvest (Kg ha^{-1})**
Sorghum		
Millet		
Maize		
Rice		
Others		

Production history of Bambara groundnut

For how long have you been growing or ever grown Bambara groundnut?

- <2 years
- 2-3 years
- 3-4 years
- 4-5 years
- >5 years

In which season (s) do you grow Bambara groundnut?

Season	
Rainy season (using rains)	
Dry season (using irrigation)	
Both seasons	

How do you grow Bambara groundnut?

Production practice	
As sole crop of seed mixtures	
Homogenous seeds of same seed coat colour	
In mixtures with other crops	
On rotation	
Others	

If in mixtures, which of the following companion crop (s) do you grow Bambara groundnut with?

Sorghum		Groundnut		Others	
Millet		Soybeans			
Maize		Tomatoes			
Rice		Pepper			
Cowpea		Onions			

If on rotation, what is/are the alternating crop(s) among the following?

Sorghum		Groundnut		Others	
Millet		Soybeans			
Maize		Tomatoes			
Rice		Pepper			
Cowpea		Onions			

What is/are the major source (s) of the Bambara groundnut seeds that you grow?

Seed acquisition	
Own size	
Neighboring farmers	
Open markets	
Seed retailers	
Government agencies	
Others	

Production and consumption

For what purpose do you produce Bambara groundnut?

Production purpose	
Home consumption	
Sell for cash	
Both home consumption and cash	
Animal feed	
Medicinal	
Socio-cultural values	
Religion	
Others	

If you consume, in what form do you consume the Bambara groundnut you produce?

Method of consumption	
Fresh pods	
Dry pods	
Both fresh and dry pods	
Processed	
Others	

If production is for sell, in what form do you sell the Bambara groundnut you produce?

Form of disposal	
Fresh pods	
Dry pods	
Both fresh and dry pods	
Processed	
Fodder for animal feed	
Others	

If you sell the Bambara groundnut produce, to whom do you sell out?

Form of disposal	
Open market	
Seed retailers	
Company	
Others	

Production and production constraints associated with Bambara groundnut

Are you currently growing Bambara groundnut?

Yes☐; or No☐.

What are your problems associated with Bambara groundnut production?

Production problems	
Lack of improved variety	
Insect pests	
Disease	
Germination	
Weeding	
Harvesting	
Yield	
Storage	
Shelling	
Rainfall	
Drought	
Soil fertility	
Cooking	
Other processing	
Market	
Lack of enough land	
Competition with other legumes	
Financial support	
Fertilizer	
Insecticides	
Fungicides	
Rodenticides	

Farmers' Bambara groundnut seed preference

What is your varietal choice for Bambara groundnut? Is it:-

Pod traits:

Pod shape	
Blunt without point	
Pointed at one end	
Pointed at both ends	
Others	
No preference	

Pod colour	
Yellowish	
Brown	
Reddish	
Purple	
Black	
Others	

Pod texture	
Smooth	
Grooved	
Folded	
Others	

Seed traits

Seed shape	
Round	
Oval	
Others	

Seed size	
Small	
Medium	
Large	

Seed feature and composition	
Pure seed colour	
Seed mixtures	

Seed colour	
Cream	
Brown	
Red	
Speckle	
Butterfly	
Seed mixtures	
Others	

Local name (s) of the landraces that you use

Name	Seed coat colour	Seed size

Other agronomic characteristics

Plant growth habit	
Erect	
Semi-erect	
spreading	

Maturity	
Early	
Medium	
Late	

Quality traits

Trait	
Taste	
Cooking time	

Any other information or comment you need to add that the questionnaire did not discuss
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