



UNIVERSITI PUTRA MALAYSIA

***INHERITANCE AND GENETIC ANALYSIS OF SEED WEIGHT AND
ASSOCIATED TRAITS IN BAMBARA GROUNDNUT
(*Vigna subterranea L.*) GENOTYPES***

KHALIQI ATIQUULLAH

FP 2021 54



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By

KHALIQI ATIQUULLAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra
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of Science

June 2021

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DEDICATIONS

My incomparable parents
and invaluable teachers in all realms of my studies.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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KHALIQI ATIQULLAH

June 2021

Chairman: Professor Mohd Rafii Yusop, PhD

Faculty: Agriculture

Knowledge of genetic variability within species of Bambara groundnut and the nature of breeding techniques are critical in this crop improvement programme. The importance of this information in underutilised crop species that mainly existing as landraces with limited information about their breeding systems and genetic diversity becomes important. Hence, this study was conducted to evaluate the genetic variability and to establish the relation between yield and its components in Bambara groundnut using multivariate analysis based on seed weight. To achieve this objective, firstly, a field trial was conducted in randomized complete block design with three replications on five Bambara groundnut genotypes from Nigeria, namely; Giwa, Duna, Cancaraki, Jatau, and Maiki. Prior to the field evaluation, the seeds of each genotype were grouped into three seed weight categories namely large, medium and small-seed size. In the second experiment, 28 lines were selected based on top grain yield plants from each seed weight category from first generation of the previous field evaluation. Data were collected on 25 agro-morphological traits and statistical analysis was conducted using SAS version 9.4 software for the analysis of variance, while variance component, genotypic and phenotypic coefficient variation, heritability and genetic advance values were estimated. Cluster analysis was performed using NT-SYS software to estimate the genetic relations among the Bambara groundnut lines. The present study revealed significant levels of variability among the genotypes, seed size categories and interaction of genotypes and seed size categories for most of the yield and yield components. High values of genotypic coefficient of variation ($GCV \geq 20\%$) coupled with genetic advance ($GA \geq 20\%$) was recorded for the number of medium seed weight per plant ($GCV=36.76\%$, $GA=36.15\%$), total large size seed weight per plant (30.34%, 28.26%), total medium size seed weight per plant (25.25%, 21.42%) and hundred seeds weight (20.02%, 29.29%), while moderate $GCV (>10\%)$ value was obtained for the total number of seed per

plant (11.34%). Among the genotype and seed size combinations, G5S is the best for yield and yield component traits, while genotypes G3 is the best for yield and yield components, whereas among the seed size categories small seed size showed the high yield and yielding components. It was concluded from the second-generation progenies evaluation indicates that considerable amount of variation exists among Bambara groundnut cultivars for yield and yield components. Moderate broad sense heritability values couple with high genetic variance for growth traits such as wide plant canopy (52.17%) and number of branches (34.36%), while low broad sense heritability values (0 to 30%) were recorded for other traits. Almost all of the traits positively associated with the yield trait except the number of days to emergency, plant height, wide canopy, days to maturity, fresh and dried biomass weight. Finally, the lines evaluated were grouped into seven primary clusters using the unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the measured traits. Based on genetic diversity results and agronomic traits performance, improved lines, G5LR1P3, G1LR1P3, G4LR1P1, G2SR1P1 and G3SR1P4 performed best in terms of yield and yield components. These improved lines are recommended for large scale evaluation and utilisation in the future breeding programme for development of high yield Bambara groundnut varieties.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**ANALISIS GENETIK DAN PEWARISAN BERAT BIJI BENIH
DAN CIRI-CIRI BERSEKUTU DI DALAM GENOTIP
KEKACANG BAMBARA (*Vigna subterranea* L.)**

Oleh

KHALIQI ATIQUULLAH

Jun 2021

Pengerusi: Profesor Mohd Rafii Yusop, PhD
Fakulti: Pertanian

Pengetahuan mengenai kepelbagaian genetik dalam spesies kekacang Bambara dan teknik-teknik pembiakbaaan adalah sangat penting dalam program pembangunan tanaman ini. Kepentingan maklumat ini di dalam spesies tanaman yang kurang digunakan terutama ianya merupakan ras daratan yang mempunyai maklumat terhad mengenai sistem pembiakan dan kepelbagaian genetik yang mana ianya adalah maklumat sangat penting. Oleh itu, kajian ini dijalankan untuk menilai kepelbagaian genetik dan untuk menentukan hubungan antara hasil dan komponen kekacang Bambara menggunakan analisis multivariat berdasarkan berat biji benih. Untuk mencapai objektif ini, pertamanya, satu percubaan lapangan telah dijalankan menggunakan rekabentuk eksperimen blok penuh rawak dengan tiga replikasi ke atas lima genotip kekacang Bambara dari Nigeria, iaitu; Giwa, Duna, Cancaraki, Jatau, dan Maiki. Sebelum penilaian lapangan dijalankan, biji benih setiap genotip dikelaskan kepada tiga kategori berat biji benih iaitu ukuran biji benih besar, sederhana dan kecil. Dalam eksperimen kedua pula, 28 titisan telah dipilih berdasarkan hasil bijian sepokok tertinggi dari setiap kategori berat biji benih dari generasi pertama penilaian lapangan tersebut. Data 25 ciri-ciri agro-morfologi telah dicerap dan analisa statistik telah dijalankan menggunakan perisian SAS versi 9.4 untuk analisa varians, serta penentuan nilai komponen varians, pekali variasi genotipik dan fenotipik, heritabiliti dan kemajuan genetik. Analisa kluster telah dijalankan dengan menggunakan perisian NT-SYS untuk menganggarkan hubungan genetik di kalangan titisan kekacang Bambara. Kajian ini telah menunjukkan tahap kepelbagaian yang signifikan di kalangan genotip, kategori saiz biji benih dan, interaksi antara genotip dan kategori saiz biji benih bagi kebanyakan ciri hasil dan komponen hasil. Nilai pekali variasi genotip ($GCV \geq 20\%$) dan ditambah dengan nilai kemajuan genetik ($GA \geq 20\%$) yang tinggi direkodkan bagi jumlah biji benih saiz sederhana sepokok, ($GCV=36.76\%$, $GA=36.15\%$) jumlah berat benih benih

saiz besar sepokok (30.34%, 28.26%) , jumlah berat biji saiz sederhana sepokok (25.25%, 21.42%) dan berat seratus biji benih (20.02%, 29.29%), sementara nilai GCV (>10%) yang sederhana adalah diperoleh untuk jumlah biji benih sepokok (11.34%). Di antara gabungan genotip dan saiz biji benih, G5S adalah yang terbaik untuk ciri-ciri hasil dan komponen hasil, sementara genotip G3 adalah yang terbaik untuk hasil dan komponen hasil, sedangkan di antara kategori saiz biji benih, didapati saiz biji benih kecil telah memberikan hasil dan komponen hasil yang tinggi. Ini disimpulkan dari penilaian progeni generasi kedua menunjukkan bahawa terdapat variasi yang tinggi di antara kultivar kekacang Bambara untuk ciri hasil dan komponen hasil.Nilai heritabiliti luas yang sederhana dengan variasi genetik yang tinggi untuk ciri-ciri pertumbuhan seperti keluasan kanopi pokok (52.17%) dan bilangan cabang (34.36%), sementara itu nilai heritabiliti luas yang rendah (0 to 30%) dicatat untuk ciri-ciri lain. Hampir kesemua ciri mempunyai perhubungan secara positif dengan ciri hasil kecuali jumlah hari bercambah, ketinggian pokok, keluasan kanopi, bilangan hari matang, berat biojisim segar dan kering. Akhirnya, titisan-titisan yang telah dinilai tersebut dikelompokkan menjadi tujuh kluster utama menggunakan kaedah tak berpemberat kumpulan berpasangan dengan min aritmetik (UPGMA) yang berdasarkan kesemua ciri-ciri yang diukur. Berdasarkan keputusan kepelbagaiannya genetik dan prestasi ciri-ciri agronomi, titisan maju G5LR1P3, G1LR1P3, G4LR1P1, G2SR1P1 dan G3SR1P4 telah menunjukkan prestasi terbaik dari segi hasil dan komponen hasil. Titisan maju kekacang Bambara ini adalah disyorkan untuk penilaian berskala besar dan digunakan dalam program pembiakbakaan dimasa hadapan bagi pembangunan varieti kekacang Bambara berhasil tinggi.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Rafii Bin Yusop, PhD

Professor

Institute of Tropical Agriculture and Food Security

Universiti Putra Malaysia

(Chairman)

Norida Binti Mazlan, PhD

Associate Professor

Institute of Tropical Agriculture

Universiti Putra Malaysia

(Member)

Mashitah Binti Jusoh, PhD

Senior Lecturer

Faculty of Agriculture

Universiti Putra Malaysia

(Member)

ZALILAH MOHD SHARIFF, PhD

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 14 October 2021

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Name and Matric No.: Khaliqi Atiqullah, GS53144

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- the research conducted and the writing of this thesis was under our supervision;
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Signature: _____

Name of Chairman
of Supervisory
Committee: Mohd Rafii Yusop

Signature: _____

Name of Member of
Supervisory
Committee: Norida Mazlan

Signature: _____

Name of Member of
Supervisory
Committee: Mashitah Jusoh

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
GCV	Genotypic Coefficient of Variation
H.I	Harvest Index
LSD	Least Significant Difference
PCV	Phenotypic Coefficient of Variation
SE	Standard Error
UPGMA	Unweighted Pair Group Method Average

CHAPTER 1

INTRODUCTION

1.1 General introduction

Global food demand is increasing due to the continuous growth in human population (Foley *et al.*, 2011). Intensive cultivation of limited numbers of available plant species is not enough to cater increased world population, hence, there is a need to focus on the underutilized crops. Bambara groundnut (*Vigna subterranea* L.Verdc.) is a tropical pulse with underground pods and is the 3rd most essential food legume after groundnut (*Arachis hypogaea* L.) and Cowpea (*Vigna unguiculata* Walp.) (Shiyam *et al.*, 2016).

Bambara groundnut is one of the underutilized crops that could be an important future crop to cushion food demand especially in Africa and Asia continent (Olukolu *et al.*, 2012). Bambara seeds are rich in protein (19%), carbohydrates (63%), fat (6.5%), and essential amino acids (Minka & Bruneteau, 2000; Amarteifio *et al.*, 2006; Shiyam *et al.*, 2016). With a more inclusive nutritional information, breeding strategies can be created for Bambara groundnut that has verified promising plants such as chickpea and soybean (Azman *et al.*, 2019).

Bambara groundnut is an underutilized crop has many agronomic potentials such as drought-tolerant and adaptation to low-nutrient climate (Goli, 1995; Zeven, 1998; Berchie *et al.*, 2012). One of the major hindrances contributing low production of Bambara groundnut is the cultivation of local landraces (Fatimah *et al.*, 2018). Thus, intensifying research in this line might be perceptive because it will be a source of cheap input and better economic paybacks (Harouna *et al.*, 2018).

The seed is the forerunner of the next generation in the life of a plant and high-quality seed is an important prerequisite for high crop yield. Only high-quality seeds will produce strong plants (FAO, 1981). The seed habit in plants has developed over a long period and represents the highest type of plant development (Stanton, 1985). The availability of high-quality seed is critical to the success of Bambara groundnut production (Chibarabada *et al.*, 2014). A seed scientist might define good-quality seed as the total of many different characteristics, such as genetic quality, physical purity, germination, vigour, size uniformity, and disease resistance (Basra 2005). To a farmer, high-quality seeds have all of the morphological, physiological, pathological, and genetic characteristics that influence eventual production (Basra, 1995).

Physiological attributes (viability and vigour) are characteristics of seeds that are connected to emergence and early crop establishment (Tekrony & Egli 1991). It is critical to assess seed quality before to planting, specifically with landraces, where seed quality is frequently unknown. Seeds that are alive and have the ability to sprout when subjected to favorable germination conditions are known as viable seeds (Basra 1995; McDonald & Copeland 1997). A number of factors influence imbibition kinetics, like seed shape, seed coat thickness, and hydration matrices (Modi, 2013). Variation in physical, physiological, and genetic seed quality characteristics of different Bambara groundnut landraces is blamed for differences in Bambara groundnut establishment outcomes (Chibarabada *et al.*, 2014).

Seed weight/size is an important agronomic trait because it is positively associated with total yield, (Kadams & Sajo, 1998). Generally, grain yield increases as the seed weight increases and vice versa. Plant breeders need genetic materials with maximum variability in selection before embarking on breeding programs on the development of superior genotypes (Pasquet & Fotso, 1991; Ndiang *et al.*, 2012; Sobda *et al.*, 2013). Seed size has a significant effect on the future performance of the seedling. Within species, larger seeds tend to germinate faster and produce larger seedlings. In general, however, if seed spacing adopted allows plants to express their full potential, larger seeds give a considerable increase in yield in comparison with smaller seeds (Forbes & Wastson, 1992). Initial drying at the higher temperature resulted in a faster reduction in seed moisture content. The effect of the different drying regimes on subsequent longevity was not significant for most accessions. Nonetheless, the seed longevity of smaller-seeded accessions was perhaps enhanced by initial drying at a higher temperature; in contrast, initial drying at 17°C appeared to be the best drying treatment for larger-seeded accessions (Jones *et al.*, 2020).

Knowledge on the genetic diversity and structure among the Bambara groundnut landraces can pave the way for the effective use and cultivation of this crop, Understanding the genetic variability of Bambara landraces will increase the efficiency of future crop improvement programs and will encourage the expansion of Bambara production areas (Ontong *et al.* 2021).

Important issue in development of Bambara groundnut local lines was a genetic diversity, so that they need to be purified in order to develop a new variety or as a parent crosses (Arif *et al.* 2016). The excessive degrees of allelic difference in Bambara groundnut germplasm could be exploited in breeding activities to develop new varieties. It is important to match recognized breeding objectives with documented diversity in order to significantly improve breeding (Muhammad *et al.* 2020). In a study conducted in Malaysia, researchers detected genetic divergence among Bambara groundnut accessions, as well as existing variety and selection for the development of high yielding pure lines for crop improvement.

As a result, the research revealed the diversity of Bambara groundnut landraces that were transferred from Africa (Nigeria) to Asia (Malaysia) (Khan et al. 2021). The capacity for successful selection is demonstrated by characters with a high genotypic coefficient of variability in genetic studies. Grain yield was described as a complex character that depends on contribution from other yield components (Kadams & Sajo, 1998). In addition to having high yield, the choice of parents having a good combination potential and a distant genetic relationship to prevent inbreeding depression is a prerequisite (Halluer et al., 2010). The availability of genetic variance can be beneficial for this crop improvement through cross breeding as well as the plant breeders to prefer desirable traits in *V. subterranea* L. Verdc. for further breeding purposes (Khan et al. 2021).

Genetic variation and the heritability of quantitative traits are among the key attentions for genetic improvement of crops (Alidu et al., 2013; Nicole et al., 2009). It was used to determine the genetic variability of Bambara groundnut accessions based on the success of morphological characters in evaluating the diversity in other crops (Ddamulira et al., 2016). Furthermore, morphological characters that can be highly correlated with grain yield give breeders the choice to decide which features to pick for crops to be evaluated (Odireleng, 2012). Characters affecting yield are inherited quantitatively and are affected by environmental interaction (Jonah et al., 2010).

Consequently, this could potentially contribute to increase farmer, especially small-scale producer's income, better food security, especially in regions of water scarcity and improve the quality of local diets within the region. This is confirmed by the self pollinating characteristics of the crop, as well as the slow gene flow identified between the diverse origins. Future recommendations would include the addition of more landraces and a more intense study on the morphological traits, because the knowledge of the genetic diversity and population structure of Bambara groundnut landraces can contribute to the cultivation and improvement of this crop. (Ontong et al. 2021). Khan et al. 2021 have claimed that the enhancement of yield and other yield-related traits of Bambara groundnut can be obtained through selection by the determination of different genetic parameters analysis. However, plant breeders may find it advantageous in the development of agromorphic features in Bambara groundnut.

1.2 Problem statement

Bambara groundnut remains one of the most neglected and underutilized grain legumes which reflects its low rank amongst other food legumes (Hillocks et al., 2012; Shiyam et al., 2016). However, the yield production of Bambara groundnut is low due to the lack of breeding programs for varietal development. Also, there is very little information available on genetic diversity on Bambara groundnut characteristics on vegetative, yield, and yield components (Mohammed et al., 2014).

Due to diverse benefits of Bambara groundnut production, little studies have been conducted in Malaysia on this food crop compared to other legumes such as sorghum, groundnut, and cowpea (Khan *et al.* 2021), , also yet, despite their nutritional importance, many (indigenous) pulses remain to be cultivated by small scale farmers and are yet to be exploited commercially as protein sources (Calles *et al.*, 2019). Furthermore, Bambara groundnut is seen as a future crop and is recognized as a Millennium crop, particularly in Malasia (Khan *et al.* 2020). Also known to withstand harsh environmental conditions, this crop can be considered as a valuable crop in the current context of sustainability and climate change (Calles *et al.*, 2019; Feldman *et al.*, 2019), thus making it an important crop to be valorized beyond its local boundaries. Nowadays, the only accessible improvement technique is to use genotype selection. However, the amount of progress that can be made through direct selection is limited by the amount of heritable variability that is available Onwubiko *et al.*, (2019).

Also inadequate knowledge of the genetic components and heritability values of Bambara groundnut based on seed weight is another major setback (Joint, 2004). So, the seed is a primordial input for crop production and it is the embodiment of past harvests as well as the promise of future ones. Little attention has been recorded on the impact of seed weight on yield in Bambara groundnut (Pasquet & Fotso, 1991; Ndiang *et al.*, 2012; Sobda *et al.*, 2013). As such there are no improved varieties of Bambara groundnut available in the major growing areas. Landraces can provide breeders with sources of genes for adaptability to different environments, nutritional characteristics and yield potential (Mohammed *et al.*, 2019). Germplasm collection and diversity analysis in different growing places such as Malaysia is one of the finest techniques to limit the hereditary erosion of the Bambara groundnut in the current context of global climate change. It could aid in the identification of currently cultivated landraces as well as the establishment of a management and upgrading system for varietal traits (Khan *et al.* 2021).

1.3 Objectives

The purpose of this research was to determine the inheritance and genetic analysis of seed weight and associated traits in Bambara groundnut genotypes. Therefore, the specific objectives of this study were:

- i) To quantify the seed weight inheritance character of five Bambara groundnut genotypes.
- ii) To estimate the genetic components, heritability and phenotypic relationship between seed weight with associated agro-morphological characters in the Bambara groundnut genotypes.
- iii) To select high yield lines based on seed weight for future breeding programs.

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BIODATA OF STUDENT

Khaliqi Atiqullah was born on 10 May 1987, in Herat Province of Afghanistan. He received his both primary and secondary school in Molina Ab.Rahman Jami High School, Herat, Afghanistan. He earned a Bachelor of Science degree in Agronomy, Agriculture Faculty Herat University, Afghanistan in 2012. Upon completion of Bachelor degree, he was admitted into a Master program in the field of Genetic and Plant Breeding, Crop Science, Agriculture Faculty in the University Putra Malaysia (UPM).



PUBLICATION

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