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UNIVERSITI PUTRA MALAYSIA  
BERILMU BERBAKTI

**YIELD IMPROVEMENT AND GENOTYPE BY ENVIRONMENT  
INTERACTION OF SELECTED BAMBARA GROUNDNUT GENOTYPES  
DEVELOPED THROUGH PURELINE SELECTION AND MOLECULAR  
APPROACHES**

By

**MD MAHMUDUL HASAN KHAN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**September 2022**

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## DEDICATIONS

*This thesis is dedicated to my parents (**Mahmuda Rahman and Md Matiur Rahman Khan**) for their boundless love, care, support, and sacrifice throughout my study life. I also dedicated it to my beloved family (Sarmin Akter, Anamta Manha, & Abdullah-al-Muhtady Khan). Likewise, I would like to devote my efforts to the people of the People's Republics of Bangladesh.*



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**September 2022**

**Chairman: Professor Mohd Rafii Yusop, PhD  
Institute: Tropical Agriculture and Food Security**

As a crop for the new millennium, Bambara groundnut (*Vigna subterranea* [L.] Verdc.) is considered as a "miracle lucrative" legume for Asia and Africa because of its drought resilience, excellent nutritional profiles, and significant versatile uses. Due to minute floral biology and cleistogamous reproductive system, its potential for improvement through hybridization breeding is limited. However, addressing genetic diversity and pure line selection, along with molecular breeding issues are the most imperative and viable methods for its improvement, as a newly introduced legume in Malaysia. The progress of this underexploited legume has been limited owing to a lack of extensive research, marginalization, inadequate knowledge, and readily available research funding, among other concerns. In this point of view, a comprehensive breeding program is carried out. The main objective of this research work was to assess morphological variation considering 14 qualitative and 27 quantitative traits as well as advancing the generation via pure-line selection, genomic DNA analysis using inter-simple sequence repeat (ISSR) markers, genotype by environment (GEI) effects on yield components, and yield stability assessment to discover the superior variety. The plant materials were sourced from the GenBank of the Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia (UPM), Serdang, Selangor. The field experiment adopted a randomized complete block design (RCBD) with three replications. The recorded data were subjected to analysis of variance, correlation, principal component analysis, clustering pattern, and heatmap analysis, as well as genetic parameters estimation. Analysis of variance revealed significant differences at  $p \leq 0.05$  or  $p \leq 0.01$  signifying a degree of variability present in evaluated traits for all generations of evaluation ( $S_0$  to  $S_5$ ). The result showed a downward trend in values of coefficient of variation for yield trait as 55.15%, 30.18%, 19.37%, 18.74%, and 17.53% for selfed generation of  $S_0$ ,  $S_1$ ,  $S_2$ ,  $S_3$ , and  $S_4$ , respectively indicating that pure line selection is effective for this legume improvement based on yield trait. Additionally, in all rounds of evaluated generation ( $S_0$ - $S_4$ ), yield component traits such as total number of pods, fresh pod weight (g), hundred seed weight (g), dry pod weight (g), dry seed weight (g), and harvest index (%) had a

positive and highly significant correlation ( $0.75 \leq r \leq 1.00$ ;  $p \leq 0.01$ ) with yield trait. Selection based on these traits for this legume improvement may lead to higher success due to their estimated high heritability and genetic gain values of  $H_b \geq 60\%$  and  $GA \geq 20\%$ , respectively. In terms of molecular characterization, a set of 32 ISSR markers on forty-four accessions from S4 selfed generation, revealed 97.64% polymorphism with an average calculated polymorphic information content (PIC) was 0.243, Nei's genetic diversity ( $h = 0.248$ ), and Shannon's information index ( $I = 0.395$ ), which indicate the presence of a low to moderate level of genetic variability among the accessions. UPMGA phylogenetic tree clustered the entire genotypes into 3 major clusters and 6 sub-clusters that were also proved by admixture (Bayesian model) analysis which assembled genotypes into 3 distinct genetic components (membership likelihood  $Q > 0.60$ ) based on best  $\Delta K = 3$ . Within the population, estimated variation was higher as 75% compared to among the population of 25% with gene flow of  $Nm = 0.9229$  indicating that higher-level genetic variation exists within the population. The total variation of 26.51% was revealed by principal component analysis (PCA) based on ISSR data, where PC1 (13.92%) > PC2 (12.59%). The path-coefficient analysis explored that dry pod weight had the highest direct effect on yield trait. GGE biplot represents the variation of the first two principal components, PC1 (94.97%) and PC2 (3.11%) for yield trait. The source of variation namely, genotypes (22.40%), seasons (31.13%), and locations (14.02%) were found to be the most significant causes of yield heterogeneity for overall  $G + E + G \times E$  variation. The GGE biplot and AMMI revealed that the three winning lines S5G1 (MaikP12-18), S5G3 (Maik P6-18), and S5G5 (JataP1-18) appear as superior lines and in addition, based on ideal genotype ranking, line MaikP12-18 (S5G1) was highly stable, with a high mean yield across the environments. Conclusively, these three superior lines namely, S5G1 (MaikP12-18), S5G3 (Maik P6-18), and S5G5 (JataP1-18) are highly recommended for further large-scale evaluation before release as newly developed varieties for commercial cultivation in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENAMBAHBAIKAN HASIL DAN INTERAKSI GENOTIP DENGAN PERSEKITARAN KE ATAS GENOTIP TERPILIH KACANG BAMBARA YANG DIBANGUNKAN MELALUI PENDEKATAN PEMILIHAN TITISAN TULEN DAN MOLEKULAR**

Oleh

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Sebagai tanaman untuk alaf baharu, kacang Bambara (*Vigna subterranea* [L.] Verdc.) dianggap sebagai kekacang “keajaiban lumayan” untuk Asia dan Afrika kerana daya tahan kemarau, profil pemakanan yang sangat baik dan kegunaan serba boleh yang signifikan. Disebabkan oleh biologi bunganya yang kecil dan sistem pembiakan kleistogami, potensinya untuk diperbaiki melalui pembiakbakaan penghidridan adalah terhad. Walau bagaimanapun, bagi menangani isu-isu kepelbagaian genetik dan pemilihan titisan tulen, bersama dengan pembiakbakaan molekul adalah kaedah yang paling penting dan berdaya maju untuk penambahbaikannya, sebagai kekacang yang baru diperkenalkan di Malaysia. Kemajuan kacang Bambara yang kurang dieksploitasi ini adalah terhad disebabkan oleh kekurangan penyelidikan yang meluas, peminggiran dan pengetahuan yang tidak mencukupi serta pembiayaan penyelidikan yang terhad, adalah antara kebimbangan yang wujud. Berdasar pandangan ini, satu program pembiakan yang komprehensif telah dijalankan. Objektif utama penyelidikan ini adalah untuk menilai variasi morfologi 14 ciri kualitatif dan 27 ciri kuantitatif serta memajukan generasi seterusnya melalui pemilihan titisan tulen, analisis DNA genomik menggunakan penanda molekul antara jujukan berulang mudah (ISSR), kesan interkasi genotip dengan persekitaran (GEI) ke atas komponen hasil, dan seterusnya penilaian kestabilan hasil untuk membangunkan varieti unggul. Bahan tanaman kajian ini diperoleh daripada GenBank Institut Pertanian Tropika dan Sekuriti Makanan (ITAFoS), Universiti Putra Malaysia (UPM), Serdang, Selangor. Eksperimen di lapangan menggunakan reka bentuk blok lengkap rawak (RCBD) dengan tiga replikasi. Data yang direkodkan tertakluk kepada analisis varians, korelasi, analisis komponen utama, corak pengklusteran dan analisis peta haba, serta penganggaran parameter genetik. Analisis varians merekodkan perbezaan ketara pada  $p \leq 0.05$  atau  $p \leq 0.01$  yang menunjukkan wujud kepelbagaian dalam ciri yang dinilai untuk semua generasi yang dinilai ( $S_0$  hingga  $S_5$ ). Keputusan merekodkan corak penurunan dalam nilai pekali variasi untuk ciri hasil iaitu masing-masing 55.15%, 30.18%, 19.37%, 18.74% dan 17.53% untuk generasi swakacuk  $S_0$ ,  $S_1$ ,  $S_2$ ,  $S_3$  dan  $S_4$ , menunjukkan bahawa pemilihan titisan tulen adalah berkesan

untuk memperbaiki tanaman ini berdasarkan ciri hasil. Tambahan pula, di dalam semua generasi yang dinilai ( $S_0$ - $S_4$ ), didapati ciri-ciri komponen hasil seperti jumlah bilangan lenggai, berat lenggai segar (g), berat seratus biji (g), berat lenggai kering (g), berat biji kering (g), dan indeks penuaian (%) mempunyai korelasi positif yang sangat signifikan ( $0.75 \leq r \leq 1.00$ ;  $p \leq 0.01$ ) dengan ciri hasil. Pemilihan berdasarkan ciri-ciri tersebut untuk pembiakbakaan kekacang ini akan memberikan kejayaan yang tinggi kerana mempunyai anggaran nilai kebolehwarisan dan kemajuan genetik tinggi iaitu masing-masing iaitu  $H_b \geq 60\%$  dan  $GA \geq 20\%$ . Dari segi pencirian molekul, satu set 32 penanda ISSR keatas empat puluh empat aksesori daripada generasi swa-kacuk  $S_4$ , menunjukkan 97.64% polimorfisme dengan anggaran purata kandungan maklumat polimorfik (PIC) adalah 0.243, kepelbagaian genetik Nei ( $h = 0.248$ ), dan Shannon indeks ( $I = 0.395$ ), yang menunjukkan kewujudan tahap kebolehubahan genetik yang rendah hingga sederhana di kalangan aksesori tersebut. Pokok filogenetik UPMGA mengklusterkan keseluruhan genotip kepada 3 kluster utama dan 6 subkluster yang turut disahkan oleh analisis *admixture* (model Bayesian) yang mengklusterkan genotip kepada 3 komponen genetik yang berbeza (kebarangkalian keahlian  $Q > 0.60$ ) berdasarkan  $\Delta K = 3$  terbaik. Dalam populasi, anggaran variasi adalah lebih tinggi iaitu 75% berbanding dengan di antara populasi iaitu 25% dengan aliran gen  $Nm = 0.9229$  menunjukkan aras variasi genetik lebih tinggi di dalam populasi ini. Dari analisis komponen utama (PCA) yang berdasarkan data ISSR, didapati jumlah variasi sebanyak 26.51% di mana PC1 (13.92%) > PC2 (12.59%). Analisis pekali laluan mendapati bahawa berat lenggai kering mempunyai kesan langsung tertinggi ke atas ciri hasil. Biplot GGE mewakili variasi dua komponen utama pertama, PC1 (94.97%) dan PC2 (3.11%) untuk ciri hasil. Sumber variasi iaitu, genotip (22.40%), musim (31.13%) dan lokasi (14.02%) didapati menjadi peyumbang heterogeniti hasil yang paling signifikan bagi variasi keseluruhan  $G + E + G \times E$ . Biplot GGE dan AMMI mendapati bahawa tiga titisan pemenang iaitu S5G1 (MaikP12-18), S5G3 (Maik P6-18) dan S5G5 (JataP1-18) muncul sebagai titisan unggul, dan tambahan pula berdasarkan kedudukan genotip ideal, titisan MaikP12-18 (S5G1) adalah sangat stabil, dengan purata hasil yang tinggi merentasi persekitaran. Secara konklusifnya, tiga titisan unggul ini iaitu, S5G1 (MaikP12-18), S5G3 (Maik P6-18), dan S5G5 (JataP1-18) adalah sangat disyorkan untuk penilaian berskala besar selanjutnya sebelum diistiharkan sebagai varieti baru yang dibangunkan untuk penanaman komersial di Malaysia.



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I certify that a Thesis Examination Committee has met on **02 September, 2022** to conduct the final examination of **Md Mahmudul Hasan Khan** on his thesis entitled “Yield improvement and genotype by environment interaction of selected Bambara groundnut (*Vigna subterranea* L. verdc.) genotypes developed through pureline selection and molecular approaches” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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## LIST OF ABBREVIATIONS

AEC	Average environment coordinate
AMMI	Additive main effect and multiplicative interaction effect
ANOVA	Analysis of variance
AOCC	African Orphan Crops Consortium
AMOVA	Analysis of molecular variance
AFLP	Amplified Fragment Length Polymorphisms
bi	Regression slope
bp	Base pairs
CCFF	Center for Crops for the Future
CV	Coefficient of variation
CTAB	Cetyltrimethylammonium bromide (C <sub>19</sub> H <sub>42</sub> BrN)
cm	Centimetre
DARWin	Dissimilarity Analysis and Representation for windows
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleoside triphosphate
DNMRT	Duncan's New Multiple Range Test
EDTA	Ethylenediaminetetraacetic acid (C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>8</sub> )
EMS	Expected mean square
F <sub>st</sub>	Fixation index
GD	Genetic distance
G <sub>st</sub>	Gene differentiation
GV & PV	Genotypic variance and phenotypic variance
GA	Genetic advance
GCV	Genotypic coefficient of variation
G×E	Genotype × environmental interaction

GGE	Genotype main effects plus genotype by environmental interaction
GGL	Genotype plus genotype by location interaction
<i>g</i>	Gram
<i>h</i>	Nei's (1973) gene diversity
$h^2b$	Broad sense heritability
ha	Hectare
$H'$	Shannon diversity index
ISSR	Inter simple sequence repeat
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
kg	Kilogram
LSD	Least significant difference
mm	Millimetre
Mg	Milligram
MVSP	Multivariate Statistical Package
$MS_E$	Mean square of error
$MS_G$	Mean square of genotype
METs	Multiple environment trials
Min	Minute
NTSYS	Numerical Taxonomy and Multivariate Analysis System
$n_e$	Effective number of alleles
Nm	Gene flow or Genetic drift
$N_a$	Number of alleles
NCSS	Number Cruncher Statistical Systems
<i>p</i>	Probability
PCV	Phenotypic coefficient of variation
PC	Principal component

PCA	Principal component analysis
PopGene	Population genetic analysis
PCR	Polymerase chain reaction
REML	Restricted Maximum Likelihood
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction fragment length polymorphism
r	Correlation of variation
S <sup>2</sup> d	Deviation from regression
SSR	Simple sequence repeat
SAS	Statistical analysis software
SASG×E	SAS genotype × environmental interaction analysis program
SVD	Singular value decomposition
SVP	Singular value partition
SVP	Singular value portioning
StD	Standard deviation
SE	Standard error
SAS	Statistical Analysis System
Taq	<i>Thermos aquaticus</i>
TBE	Tris EDTA buffer
Tm	Melting temperature
Ta	Annealing temperature
UV	Ultraviolet
UPMGA	Unweighted pair group method with arithmetic mean
VERCOM	Variance component
WHC	Ward hierarchal clustering
Wi	Wricke's ecovalence
YSi	Kang's yield stability statistics

$\beta_i$	Perkins and Jinks beta
$\sigma^2$	Shukla's variance
$\mu\text{g}$	Microgram
$\mu\text{l}$	Microliter
$\mu\text{m}$	Micrometre
$\Sigma\text{RSI}$	Rank summation index
%	Percentages
$^{\circ}\text{C}$	Degree Celsius



# CHAPTER 1

## INTRODUCTION

### 1.1 General introduction

Bambara groundnut (*Vigna subterranea* [L.] Verdc. Syn. *Voandzeia subterranea* [L.] Thouars ex DC.  $2n = 2x = 22$ ) belongs to an important taxon: the genus *Vigna*, under the member of Fabaceae or Leguminosae family and Faboideae subfamily (Heller et al., 1997). Bambara groundnut is widely cultivated as a subsistence crop, however, after peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) it becomes Africa's third most prevalent legume in terms of consumption and production (Obidiebube et al., 2020). The crop contributes to increasing soil fertility profile by fixing atmospheric nitrogen and can withstand drought as well as thrive in poor soils where other legumes fail to survive (Minnaar-Ontong et al., 2021). Even though it is a consistent source of food for low-income people and sources of earning by selling excess Bambara groundnut regularly in local markets, as well as offering a good source of essential nutrients (Olanrewaju et al., 2021a). Consumption of agri-based food reduces mortality, resulting in coronary heart diseases so, Bambara groundnut can be an agri-based protein source for marginal people who are unaffordable to precious animal protein (Oludare et al., 2017). Bambara groundnut is a hardy crop that has been noted as a lucrative and nutritive food source when other food is under threat (Mbosso et al., 2020). Due to balanced nutritional profiles viz. carbohydrates (64.4%), protein (23.6%), oil (6.5%), fibre (5.5%), and a significant amount of minerals it is remarked as a “Complete Food” having the potentiality to mitigate nutritional gaps and ensure food safety (Lin Tan et al., 2020).

The main drawback of expansion of this legume cultivation is limited yield potential, which can be overcome by neglecting native cultivars with replacing the high-yielding modern cultivars through comprehensive research (Obidiebube et al., 2020). Evidence on the genetic relationship of a crop considering molecular and morphological markers is imperative for effective breeding schemes (Fatimah and Ardiarini, 2018). In literature, negligible evidence was found to use of ISSRs marker in Bambara groundnut. To identify genetically similar lines, ISSRs have potential by generating very repeatable and authentic bands on evaluated genotypes (Nilkanta et al., 2017). A set of stability statistical measures namely biplots (AMMI and GGE) are widely executed in different crops to graphically show the interrelationships between genotypes (G), environments (E), and GEI as well as for detecting the superior stable genotypes over environments (Olanrewaju et al., 2021b). Several studies, including Olanrewaju et al., (2021b) and Mogale, (2018), have successfully established GGE biplots-based genotype assessment to assess yield stability.

However, as a new crop in Malaysia, a gap in research at the genomic level, information on the crop's botany, modern farming techniques, economic value addition, and diversity of *V. subterranea* prompted the inauguration of this research.

## **1.2 Problems statement**

Bambara groundnut is a highly nutritious and potentially lucrative crop in Malaysia, having the potential to provide a significant impact on world food and nutritional security (Belel, 2018). Globally, this under-researched legume received little attention by researchers and policymakers compared to the other pulses and legumes for its ultimate improvement (Muhammad et al., 2020). Despite its great yield potential, its existing yield is unimpressive, owing to a lack of systematic genetic improvement (Majola et al., 2021). The main point of low yield is the use of local landraces in addition to little or no interest in its genetic improvement, lack of improved varieties, traditional production technologies, lack of effective research and resources, less research by scientific personnel (Mohammed, 2019).

Due to cleistogamous floral behaviour, numerous attempts at hybridization have failed horribly, with fewer than 2% success (Muhammad et al., 2020). No high-yielding cultivars have been released yet in Malaysia. However, this legume is successfully grown in some pockets area of Sik district Kedah, Malaysia using local cultivars. Little is known about the relationship between yield and its aiding traits, though, it was definitely insufficient to assist breeders in selecting successful cultivars. Despite the fact that numerous attempts have been made on pure line selection, molecular characterization, and GEI assessment in various legumes, however, a little or no work has been executed on this underexploited legume in Malaysia. Therefore, it is imperative to achieve genotypes that displayed outstanding performance in grain yield that can be used as breeding lines for the genetic improvement of the Bambara groundnut.

## **1.3 Significance of the study**

The ever-increasing demand for food and nutrient consumption justifies the need to increase current yield by introducing high-yielding cultivars. So, this study focuses on the genetic divergence in various qualitative and quantitative variables to investigate the diversity across the genotypes and variables, subject to the selection of elite homogenous lines for future breeding programs with a specific goal of high yield. This research will be a significant endeavour toward promoting the Bambara groundnut molecular breeding strategy in Malaysia. Moreover, the current study will provide an idea of the efficiency of different stability analysis methods for selecting high-yielding superior and stable genotypes over a wide range of environments. Additionally, a better understanding of their morphological and molecular genetic relationship, as well as G×E interaction effects assessment will be beneficial for judicial utilization, maintenance, conservation, application of limited genetic resources.

#### 1.4 Research hypothesis

The study hypothesised that, the research findings will deliver significantly unique *V. subterranea* genetic materials that can be announced as superior variety for large-scale commercial farming in different agroecological zone of Malaysia.

#### 1.5 Research objectives

##### **Main objective:**

To develop pure-lines and high-yielding superior stable genotypes of Bambara groundnut for commercial cultivation in Malaysia.

##### **Specific objectives:**

- i. To evaluate genetic and agronomic performance as well as advancing the generation for development of high yielding homogenous lines through pure-line selection method.
- ii. To insight into molecular diversity, phylogenetic relationship, and admixture mapping (genetic structure) of selected lines through ISSR markers system.
- iii. To study the genotype  $\times$  environment interaction effect (GEI) on yield and yield components in addition to criteria selection for crop yield improvement.
- iv. To quantify the direct and indirect effects of growth and yield components on crop yield through the degree and nature of traits association as well as path-coefficient analysis.
- v. To identify the high-yielding superior and stable accessions using univariate and multivariate stability statistical approaches over multi-environment trials.



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