# ADAPTABILITY STUDIES OF INTRODUCED MUNGBEAN (*Vigna radiata L. Wilczek*) GENOTYPES IN NIGERIA

BY

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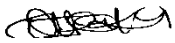
## DECLARATION

I hereby declare that except for a reference to other peoples' work, which has been duly cited, this thesis is a result of my original findings and has neither in whole nor part, been presented for a degree in Ghana or elsewhere.



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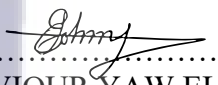

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## ABSTRACT

Mungbean (*Vigna radiata* L. Wilczek) is a nutrient-rich legume with significant potential for improving food security, soil fertility, and agricultural sustainability in Nigeria. However, its adaptability and genetic variability within the country's diverse agro-ecological zones remain largely unexplored. This study assessed the phenotypic diversity, genetic variation, and environmental adaptability of 120 introduced mungbean genotypes across four distinct environments in Nigeria: Awka (southeastern Nigeria) and Uyo (south-south Nigeria) during both dry and rainy seasons (Awka (dry and rainy seasons) and Uyo (dry and rainy seasons)). The research employed a multi-faceted approach, integrating phenotypic characterization, statistical modelling, and molecular analysis using Diversity Arrays Technology Sequencing (DArTseq). Seventeen morphological traits were evaluated to assess phenotypic variation, with Principal Component Analysis (PCA) explaining over 70% of total variance. Genotypic characterization identified 5,037 high-quality Single Nucleotide Polymorphisms (SNPs) distributed across 11 chromosomes, with chromosome 1 having the highest SNP density (689 SNPs, 13.68%). Genome-wide association studies (GWAS) were conducted using five genetic models within the multi-random mixed linear model (mrMLM) approach: pLARmEB (Polygenic-background-control-based least angle regression plus empirical Bayes), FASTmrMLM (Fast multi-locus random-SNP-effect Mixed Linear Model), FASTmrEMMA (Efficient Mixed Model Association), ISIS EM-BLASSO (Iterative Sure Independence Screening Extended Bayesian LASSO), pKWmEB (Polygenic-background-control-based Kruskal-Wallis empirical Bayes). These analyses revealed significant Marker-Trait Associations (MTAs) for yield and protein content, with a total revealed a total of 16 significant marker-trait associations (MTAs) for yield and 10 MTAs for protein content across the four environments detected. The markers associated with yield were distributed on chromosomes 2, 3, 4, 5, 6, 8, and 9, while protein content markers were found on chromosomes 1, 3, 5, and 6.

Stability analysis using Additive Main Effect and Multiplicative Interaction (AMMI) and Genotype plus Genotype-Environment (GGE) biplot models identified genotypes 130, 105, and 20 as the most stable for yield, while genotype 130 exhibited superior protein content stability. Multi-location trials further confirmed that Uyo (rainy season) was the most favorable environment for optimizing both yield and protein accumulation. These findings provide a comprehensive framework for mungbean breeding in Nigeria, highlighting the genetic potential of specific genotypes for adaptation to diverse climatic conditions. The integration of phenotypic, genotypic, and stability analyses offers crucial insights for marker-assisted selection, aiding in the development of high-yielding, climate-resilient mungbean varieties. This research lays a foundation for enhancing smallholder farmer productivity, soil fertility, and dietary diversity, aligning with national and global efforts toward sustainable agriculture and food security. These findings provide valuable insights into the genetic control of key agronomic traits, laying a foundation for marker-assisted selection in mungbean breeding programs. This study highlights the potential of mungbean to enhance food security, soil fertility, and nutritional balance in Nigeria's diverse agro-ecological zones.

## DEDICATION

I dedicate this work to my husband, children, my mother and late father

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This thesis is a culmination of collective efforts and the grace of God, and I dedicate it to all those who believed in me and supported me through this transformative journey. Thank you for being part of my story.

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# CHAPTER ONE

## 1.0 GENERAL INTRODUCTION

In recent years, the quest for sustainable agricultural practices has propelled nations worldwide to explore novel crop varieties, enhancing both productivity and resilience in the face of shifting environmental dynamics (Adhikari *et al*., 2018). In this pursuit, the introduction of mungbean (*Vigna radiata* L. Wilczek), known for its nutritional value, adaptability, and relatively short growth cycle, mungbean presents a promising opportunity to diversify and fortify the Nigeria's agricultural sector (Singh et al., 2021). Mungbean (Vigna radiata L.) is one of the most valuable pulse crops that are eaten and relished by most households in different parts of the world (Shanmugasundaram *et al*., 2009). Mungbean originated in Asia but is widely grown in Africa, Latin America, and Asia (Tomooka *et al*., 1992). Mungbean is commonly known as green gram or golden gram in many parts of the world and forms a part of the much-needed diverse grain-based diet of the poor (Cao et al., 2012). A greater portion (90%) of the world's mungbean production is in Asian countries like India, China, Pakistan, and Thailand, where 50% of the species' world production is located (Lambrides and Godwin, 2006; Senthil *et al*., 2011).

The successful integration of any introduced crop hinges on its ability to acclimate to the local environment, withstand prevalent stressors, and deliver consistent yields (Lavorel *et al.,* 2015). Understanding the adaptability of mungbean to Nigeria's diverse agro-ecological zones, soil types, and climatic conditions is paramount to it being adopted by farmers (Diatta, 2020).

Mungbean is believed to have been domesticated in India approximately 3500 years ago (Fuller and Harvey, 2006; Jain and Mehra, 1980; Singh *et al*., 1975; Vishnu Mittre, 1974). However, the wild form of mungbean, Vigna radiata var. sublobata, is indigenous to the subtropical and tropical regions of northern and eastern Australia and is widely distributed throughout Africa, Asia, and Australia (Lawn and Cottrell, 1988). Studies of protein variation and enzyme diversity reveal that mungbean in West Asia exhibits the greatest variation, suggesting that it spread to other Asian countries and Africa (Tomooka *et al*., 1992a; Dela Vina and Tomooka,

1994). Modern mungbean cultivars have resulted from multiple rounds of domestication and are currently distributed throughout southern and eastern Asia, Africa, and Austronesia (Lambrides and Godwin, 2007).



Institutions involved in mungbean germplasm conservation include AVRDC—The World Vegetable Center, Taiwan; National Bureau of Genetic Resources of the Indian Council of Agricultural Research; the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; the Plant Genetic Resources Conservation Unit of the University of Georgia, USA; and the University of the Philippines. Additionally, the Rural Development Administration (RDA), Korea, and the University of the Philippines house duplicates of some of the mungbean germplasm found in AVRDC (Ebert, 2013; Kim *et al*., 2015).

These collections are crucial for breeding programs and research as they provide a manageable yet comprehensive set of genetic resources for various studies. They act as gateways to the germplasm, facilitating the utilization of genetic resources in crop improvement and conservation efforts. Additionally, mungbean core collections have been set up in China, India, Korea, and the USA to facilitate the efficient use of genetic resources by breeders and researchers and to provide them with easier access to useful germplasm resources.

Studies have shown that the rainforest agro-ecological zone of Nigeria is well suited for the cultivation of mungbean (Agugo, 2003; Agugo and Muoneke, 2009). The short growth duration of mungbean makes it easily adaptable to different cropping systems and rotations, thus creating diversity in cropping systems (Shanmugasundaram *et al*., 2009). The nitrogen-fixing ability of mungbean has given it the ability to fertilize the soil (Sharma et al., 1996), and it requires low soil water and fertility for its production, which in turn increases cropping system productivity and resilience (Ahmad et al., 2001; Keatinge *et al.,* 2011).

Mungbean constitutes an essential component of global agriculture due to its nutritional value, adaptability, and contribution to sustainable cropping systems (Sehrawat *et al*., 2021). As a vital leguminous crop, mungbean offers multiple benefits, including nitrogen fixation, soil improvement, and dietary protein enrichment (Kumar *et al*., 2021). Despite its significance, comprehensive studies assessing the adaptability of mungbean across diverse agro-ecological zones remain relatively scarce, particularly within the context of Nigeria.

The cultivation of mungbean holds substantial promise for bolstering food security, enhancing soil fertility, and supporting the livelihoods of smallholder farmers in Nigeria. However, a profound understanding of its adaptability to various environmental conditions is pivotal for maximizing its potential contributions to the country's agricultural landscape. In Nigeria, where agriculture plays a crucial role in the economy and food production, the exploration of adaptable crops such as mungbean becomes imperative, especially considering the country's diverse agro-climatic zones, ranging from arid to humid regions. Understanding the adaptability of mungbean across these zones holds the key to leveraging its potential as a sustainable crop option and addressing food insecurity challenges prevalent in the country. Mungbean can play a major role in the eradication of protein deficiency and other mineral nutrient deficiencies in the human populations of Nigeria (Keatinge *et al*., 2011).

Baraki *et al.* (2020) reported significant genotype (G), environment (E), and genotype × environment (G × E) interactions for mungbean grain yield. Similar findings have been noted by Asfaw *et al*. (2012) and Tong et al. (2024). Kumar *et al*. (2021) highlighted that G×E interactions significantly influence mungbean yield stability in South Asian environments, emphasizing the importance of selecting stable genotypes for diverse conditions. Furthermore, studies by Wang *et al*. (2022) explored G×E impacts on yield and resilience under abiotic stresses, providing insights into genotype selection.

Research by Devi *et al*. (2020) demonstrated considerable genetic and phenotypic variability among mungbean germplasm in India, with heritability estimates aiding in targeted breeding strategies. In Nigeria, Akinyosoye et al. (2021) analyzed phenotypic variability and reported promising lines for yield and stress tolerance. Agbolade *et al*. (2022) validated similar observations, highlighting the genetic potential of mungbean in various agro-ecological zones of the country.

QTL mapping studies, such as those conducted by Singh *et al*. (2023), have identified key genomic regions linked to yield and protein content in mungbean. These findings provide a foundation for marker-assisted selection, enabling the development of high-yielding, protein-rich genotypes. Relationships between proximate composition, particularly protein, and seed yield have also been explored by Nadathur *et al.* (2017), indicating potential trade-offs and synergies crucial for breeding programs.

Mungbean (Vigna radiata L. Wilczek), renowned for its high nutritional value and nitrogen-fixing capabilities, offers substantial potential to enhance food security and soil fertility in Nigeria (Singh *et al*., 2021). Despite these benefits, the cultivation of mungbean remains limited in the country, primarily due to the lack of comprehensive adaptability studies across its diverse agro-ecological zones (Ngwuta *et al.,* 2010).

Nigeria's agricultural landscape is characterized by varied climatic and soil conditions, which present significant challenges for the introduction and successful cultivation of mungbean (Diatta, 2020). Factors such as temperature fluctuations, soil fertility, rainfall patterns, and pest and disease prevalence can markedly influence the growth, yield, and resilience of mungbean genotypes (Lavorel *et al*., 2015). Additionally, smallholder farmers, who dominate Nigeria's agricultural sector, often have limited access to mungbean varieties that are specifically adapted to local environmental stresses and capable of producing high yields (Adhikari *et al.,* 2018).

Given the promising benefits of mungbean for improving nutrition and soil health, it is crucial to conduct thorough adaptability studies to identify genotypes that can perform well in Nigeria's diverse agro-ecological zones. Such studies are essential to understand the interaction between mungbean genotypes and the local environment, thereby informing breeding programs and agricultural practices that can enhance mungbean productivity and resilience (Olum *et al.,* 2020).

Mungbean can play a major role in the eradication of protein deficiency and that of other mineral nutrients in human populations of Nigeria. (Keatinge *et al.,*2011). There is paucity of information on adaptation, yield stability and genetic studies of mungbean in Nigeria. (Akinyosoye *et al*.,2021). Mungbean is a nutrient-rich, short duration, low input crop which has shown potentials of high yield in parts of Northern and Southern Nigeria, (Agbolade *et a*l.,2022). These claims need to be validated through adaptability and stability studies. There is increased demand for diverse sources of plant protein in the diet of the teaming population of the people of Nigeria (Nadathur *et al*.,2017)

The scarcity of data on the adaptability of mungbean in Nigeria poses a significant barrier to its widespread adoption and optimal cultivation and breeding works. Studies such as those by Akinyosoye *et al*. (2021) and

Agbolade *et al*. (2022) highlight the need for targeted adaptability assessments in various agro-ecological zones. Additionally, the work of Kumar *et al.* (2021) and Wang *et al*. (2022) underscores the importance of investigating genotype-environment interactions and stress tolerance mechanisms, which remain underexplored in Nigeria. Expanding these research areas will provide valuable insights to bridge the existing knowledge gaps. Systematic research on growth rates, yield potential, pest and disease resistance, and environmental adaptability of different genotypes can provide actionable insights for policymakers, agricultural extension agencies, and farmers.



The main goal is to integrate mungbean into the food production system of Nigeria to diversify and improve availability of highly productive and nutritious foods and feed.

The specific objectives were to:

a) assess phenotypic variability of morphological traits of mungbean germplasm introduced in Nigeria;

b) assess genotype-environment interaction for seed yield and related traits;

c) assess genotypic variability of selected mungbean genotypes through sequencing; and

d) identify SNPs associated with yield and protein content of mungbean.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Mungbean- Origin, Distribution and Production

Mungbean is said to belong to the genus Vigna which is made up of about one hundred and fifty species; 22 species from India while 16 from Southeast Asia with the largest number of species being of African origin. It is diploid with chromosome number (2n=2x=22) and the genome size is quite small having about; 0.60 pg /1C (579 Mbp) (Somta and Srinives 2007). Central Asia region is thought to be the primary center of diversity of mungbean and Indian subcontinent serving the gene center and possibly location where it was domesticated and distributed. Studies have shown that mungbean was a product of a cross between wild *Vigna radiata.var. sublobata* and *Vigna radiata.var. radiata* (Shanmugasundaram, 1988). The fact that mungbean has the highest diversity in southeast Asia has been supported by a molecular study carried out using over 415 cultivated (V. radiata var. radiata), 19 SSR primers and 189 accessions (*V. radiata var. sublobata*) from the wild and 11 intermediate accessions from several geographic locations and this a further confirmation to the report of Somta and Srinives (2007) that the center of domestication of mungbean is India. About 5,900 accessions are maintained at the Asian world vegetable center (AVRDC) and this seems to be one of the largest mungbean germplasm collections around the world (Shanmugasundaram et al. 2009). It was established in 1971 and since then has functioned as major center for genetic improvement of mungbean.

The global average yield of mungbean is low at around 1,150 kg/ha (Nair & Schreinemachers, 2020) . Major challenges affecting yield include pest and disease pressures, environmental stresses, and limited investment in research and development, especially in genomics and breeding programs. Efforts are ongoing to develop improved mungbean varieties through marker-assisted and genomics-assisted breeding, which are expected to enhance yield and quality, and provide resistance to various stresses (Somta *et al*., 2022).

For the Asian region India is the largest producer of mungbean, cultivating approximately 4.5 million hectares with an average yield of about 1,200kg/ha due to various biotic and abiotic stresses (Pratap *et al*., 2019), Myanmar, the second-largest producer and the biggest exporter, cultivates mungbean on around 1.2 million hectares with an average yield of approximately 1,150 kg/ha (Somta *et al*., 2022). In China, mungbean is cultivated on a smaller scale compared to India and Myanmar, with an average yield of about 1,100 kg/ha. Thailand also contributes significantly to global mungbean production, with an average yield similar to that of China, around 1,100 kg/ha (Somta *et al*., 2022) . America has seen increasing interest in mungbean cultivation due to rising demand for plant-based proteins. However, the average yield in North America is lower compared to leading Asian countries, ranging between 800 and 1,000 kg/ha Australia is emerging as a significant producer with increasing acreage dedicated to mungbean cultivation. The average yield in Australia is approximately 1,000 kg/ha, benefiting from modern agricultural practices and favorable growing conditions ((Nair& Schreinemachers, 2020). In Africa, mungbean is gaining popularity due to its adaptability to arid regions and its nutritional benefits. Yields in Africa vary widely but are generally lower than in Asia, averaging around 600-800 kg/ha (Somta *et al*., 2022).

**2.2 Nutritional Benefits of Mungbean**

Mungbean (*Vigna radiata L. Wilczek*) is a nutrient-dense legume containing approximately 22–28% protein, 55–65% carbohydrates, and 1–2% fat. It provides a rich source of essential amino acids**,** minerals (Fe, Ca, Mg, Zn)**,** and vitamins (particularly folate and B-complex vitamins)**.** Its high digestibility and low flatulence factors make it a superior legume for improving nutritional quality and food security, especially in protein-deficient regions (Nair et al., 2021)



Consumption of plant-based diets to prevent chronic diseases and to enhance general well-being of humans has been recommended by many health organizations (Zhu Yi-Shen *et al.,*2018). This has led to the introduction of diverse functional plant- based foods into health care program of many countries (Epsin *et al*.,2007). One of these recommended crops with health benefit is the nutrient rich short duration mungbean

(*Vigna radiata*) which is a major source of essential amino acid especially lysine and protein as well (Kulsum *et al.,* 2007). Mungbean can be said to be more nutritious than other legumes because it is highly digestible and causes less flatulence more so its consumption can improve the plasma lipid profile by normalizing insulin sensitivity (Fery, 2002; Tachibana *et al* 2013). Studies have shown that mungbean is a good source of plant protein, carbohydrate, and minerals (Hussain *et al.,* 2011; Blessing and Gregory, 2010). Agugo and Onimawo (2009) reported high level of protein content in mungbean seeds as influenced by different methods of processing, this shows that its protein isolates can be made easily. The protein isolate can be used as nutrient supplements to fight protein malnutrition which is a major challenge in many developing countries where the cost of animal proteins as compared to plant protein is quite high hence many low-income earners depend more on plant protein to meet their daily protein need (Butt and Batool, 2010;). Anwar *et al*. (2007) mentioned that mungbean can significantly enrich our diets with protein, carbohydrate and a range of micronutrients in diets (Anwar *et al*., 2007). Mungbean sprout which serve as a cheap source of vitamins and minerals can be easily produced and consumed at subsistent or commercial basis at any time of the year, (Zhang and Lim, 2014). They do not contain cholesterol, about 80.00 kcal, 3.00 g of protein, 6.00 g carbohydrates, 2.00 mg of iron and only 0.20 g of fat and high amount of fiber can be gotten from consuming a cup of mungbean sprouts. Consumption of mungbean sprouts supplies high concentration of enzymes which enhances digestive process in humans. The deficiency of lysine an essential amino acid in most cereal based diet can be supplemented by preparing it with mungbean (Suresh, *et al*., 2010; Baskaran, *et al.,* 2009). Cao *et al* (2012) reported the absence of most of the anti-nutritional factors which are commonly found in cowpea and other legumes in this crop. Mungbean immature pods are usually consumed as vegetables in most rural communities, which supply the daily needed vitamins and minerals to their diets. Moreover rice-mungbean combination is easily digested at a rate (84.4%) compared to rice-meat combination in the diet of little children and this can readily meet the protein needs of humans (Hussain *et al.,* 2013). Ali *et al. (*2016) recommended the use of mungbean as a supplement for the preparation of weaning formulas for infant as a result of the amount of protein in the seeds and its hypo allergic properties. The plant proteins supplied by mungbean helps to minimize the use of land space and greenhouse gas emissions when compared to the animal-source proteins, hence achieving a better balance between dietary habits and environmental protection (Di, 2017). Other uses of mungbean includes;

being boiled and eaten as beans, it can be used to make soup or mungbean pancake (Abbas, *et al*., 2010; Gwag, *et al*., 2006). The whole mungbean plant is also useful to livestock production as the stalks, leaves and husks are used in animal feed formulation. And can also be ploughed into the soil to improve soil health (Itoh T *et al.,* 2006).



**2.3 Historical Context of Mungbean Introduction in Nigeria**

Ha and Lee, (2019) reported that the introduction of Mungbean (Vigna radiata L. Wilczek) to Nigeria represents a significant chapter in the country's agricultural history, marked by efforts to diversify crops, enhance food security, and promote sustainable farming practices. While the precise timeline of Mungbean's arrival in Nigeria may vary, its introduction can be traced back to initiatives aimed at harnessing the potential of exotic legumes for local agricultural development (Wani *et al*., 2021). The introduction of Mungbean to Nigeria occurred within the broader context of agricultural experimentation and technology transfer during the mid-20th century. Influenced by advancements in agricultural science and the quest for crop diversification, Nigerian agricultural researchers and extension agents began exploring the feasibility of introducing new crop species to complement traditional staples (Mapfumo *et al.,* 2013). Agricultural research institutions played a pivotal role in facilitating the introduction of Mungbean to Nigeria. Collaborative efforts between international organizations, such as the Food and Agriculture Organization (FAO) and the International Institute of Tropical Agriculture (IITA), and local research institutions provided platforms for evaluating the agronomic potential and adaptability of Mungbean to Nigerian agro-ecological conditions (Ashe, 2019). The process of introducing Mungbean to Nigeria involved rigorous evaluation and adaptation trials to assess its performance under diverse environmental settings (Rahman, 2022). Researchers conducted field experiments to determine suitable planting dates, optimal agronomic practices, and potential challenges associated with Mungbean cultivation in different regions of the country (Tofa *et al*., 2020). Following successful trials and positive outcomes, efforts were made to disseminate knowledge and propagate Mungbean cultivation among Nigerian farmers. Extension services, farmer training programs, and demonstration plots were utilized to promote awareness and encourage the adoption of Mungbean as a viable alternative or complementary crop to existing staples (Gloaguen *et al*., 2018). The introduction of Mungbean to Nigeria has

left a lasting impact on the country's agricultural landscape. Beyond its immediate contributions to crop diversification and food security, Mungbean cultivation has stimulated research and innovation in legume agronomy, soil management, and sustainable farming practices. Moreover, its integration into local cropping systems has enhanced resilience against environmental challenges and contributed to rural livelihoods (Amede *et al*., 2023).

### 2.4 Mungbean Cultivation in Nigeria

In Nigeria, agriculture serves as a backbone of the economy, employing a significant portion of the population and contributing substantially to the nation's Gross Domestic Product (GDP) (NBS, 2021). However, the country faces persistent challenges related to food security, sustainable crop production, and climate change impacts, emphasizing the critical need to diversify and optimize agricultural practices (FAO, 2019).

Mungbean has garnered attention as a potential solution due to its adaptability and ability to thrive in various agro-climatic conditions. Despite its potential, comprehensive studies investigating the adaptability of mungbean to specific ecological zones within Nigeria are limited (Mohammed *et al*., 2018). Existing literature primarily focuses on general cultivation practices and nutritional aspects, lacking in-depth analyses of its performance and adaptability across different regions of Nigeria.

### 2.5 Significance and Potential for production of Mungbean in Nigeria

Mungbean (*Vigna radiata* L. Wilczek) is a short-duration, self-pollinated legume crop with wide adaptability and considerable potential to contribute to food and nutritional security in Nigeria (Odeku *et al.,* 2024; Obasi *et al.,* 2024). The crop is characterized by its high protein content (22–28%), moderate carbohydrates (55–65%), low fat (1–2%), and substantial quantities of essential minerals such as iron, calcium, phosphorus, and magnesium (Iqbal *et al*., 2006; Tang *et al*., 2014; Nair *et al.,* 2021). These attributes make mungbean a valuable dietary supplement in regions with high incidences of protein-energy malnutrition and micronutrient deficiencies. Incorporation of mungbean into traditional diets could therefore play a crucial role in improving the nutritional status of vulnerable populations in Nigeria (Adelabu and Franke, 2023). Agronomically, mungbean possesses several traits that make it suitable for sustainable intensification within Nigeria's diverse agro-ecological zones. Its short growth cycle (60–75 days) allows for multiple cropping and efficient land use,

either as a sole crop or in rotation with cereals such as maize and sorghum (Altieri et al., 2015). As a legume, it forms symbiotic associations with *Rhizobium* species, enabling biological nitrogen fixation of approximately 30–90 kg N $ha^{-1}$ (Sun et al., 2019). This process improves soil fertility and reduces the dependence on synthetic nitrogen fertilizers, thereby contributing to environmentally sustainable crop production systems (Tripathi *et al.*, 2020). From an economic perspective, mungbean offers potential as a diversification and income-generating crop for smallholder farmers. Its relatively low input requirements, combined with growing domestic and international demand, make it an economically viable crop for integration into Nigeria's farming systems (Vanlauwe *et al*., 2014). Additionally, its use in value-added food and feed products—such as sprouts, flour blends, and protein isolates provides opportunities for agro-industrial development and rural employment. Ecophysiologically, mungbean exhibits tolerance to abiotic stresses such as high temperature and intermittent drought, making it particularly suited to the variable rainfall patterns observed across sub-Saharan Africa (Altieri *et al*., 2015; Aremu *et al.,* 2021). These adaptive traits enhance its potential as a resilient crop under climate change scenarios. Although mungbean is cultivated in several African countries, it remains a relatively minor crop across the continent (Mogotsi, 2006). Its introduction into Nigeria is recent but expanding, with successful production reported in Abia, Anambra, Plateau, and some northern states (Douglas *et al.,* 1982). Simulation studies conducted at the research farm of Michael Okpara University of Agriculture, Umudike, indicated a potential seed yield of 3.5 t $ha^{-1}$ under optimal management conditions (Agugo, 2010). Preliminary field trials in the same location, however, recorded an average yield of 0.46 t $ha^{-1}$, while Uko *et al.* (2019) reported 0.82 t $ha^{-1}$ when 5 t $ha^{-1}$ of oil palm bunch and poultry manure were applied as soil amendments. These yield levels are comparable to those obtained in farmers' fields in Asia (0.49 t $ha^{-1}$) (Haqqani, 1989), demonstrating the potential of mungbean to become a viable crop for Nigeria's legume production systems once adapted to local conditions.



### 2.5.1 Feasibility of Mungbean Breeding Program in Nigeria

Mungbean (Vigna radiata L.) is an underutilized pulse crop in Nigeria with significant potential to contribute to food security, nutrition, and sustainable agriculture. The introduction of mungbean in Southeast Nigeria dates back to the early 2000s, yet its production remains limited due to low awareness and minimal genetic

improvement efforts (Ukwu *et al.*, 2023). The crop is a rich source of plant-based protein, essential amino acids, vitamins, minerals, and antioxidants, offering therapeutic and environmental benefits such as nitrogen fixation and soil fertility enhancement (Ukwu *et al*., 2023; Tang *et al.*, 2014). Studies conducted on mungbean accessions in the derived savanna zone of Southeast Nigeria have revealed substantial morphological and agronomic variability among genotypes. Evaluation of ten genotypes sourced from the International Institute of Tropical Agriculture (IITA) showed significant variation in traits such as number of pods per plant, number of seeds per pod, seed yield, and flowering time (Ukwu *et al*., 2023). Accessions Tvr18 and Tvr79 emerged as the best performing genotypes with the highest yield components, suggesting their suitability as parental lines in breeding programs. Additionally, accession Tvr83 showed unique traits such as high leaf number and branching, making it a valuable candidate for hybridization to improve yield-related traits (Ukwu *et al*., 2023). In southwestern Nigeria, the adaptability and yield performance of fifteen mungbean cultivars from the World Vegetable Centre were evaluated, confirming the crop's potential under Nigerian agro-ecological conditions (Akintoye *et al.,* 2022). Among these, cultivar AVMU 1657 demonstrated superior growth and yield parameters despite later flowering. Correlation analyses indicated strong positive relationships between pod weight, pod length, and seed yield, highlighting key traits for selection in breeding programs (Akintoye *et al*., 2022). Despite its minor status and limited funding, mungbean research internationally and within Nigeria is gaining momentum due to the crop's economic and nutritional importance. Promoting genetic diversity, selection of high-yielding and early maturing genotypes, and multi-location trials are critical steps to enhance mungbean productivity and farmer adoption in Nigeria (Ukwu *et al.,* 2023; Akintoye *et a*l., 2022). Such breeding efforts align with the United Nations' Sustainable Development Goals aimed at eradicating hunger and malnutrition by 2030. Empirical evidence supports the feasibility and relevance of a mungbean breeding program in Nigeria. The available germplasm exhibits sufficient genetic variability and yield potential. Proper characterization and selection, hybridization strategies, and multi-location testing could lead to the release of improved cultivars suited to Nigerian environments, contributing to sustainable agricultural development and food security.

## 2.6 Climatic and edaphic Requirements for Mungbean Cultivation



### 2.6.1 Temperature

Mung bean is a warm season crop which can grow under a wide range of climatic condition. Mungbean has been found to perform best under temperature range of about 20°C - 30°C (Chadha, 2010). It is usually grown during summer. Mungbean respond well to long day length, different varieties vary in the way they respond to it when exposed to short day length they tend to flower earlier but the reverse is the case when exposed to long day length. They are however generally considered as heat and drought tolerant (DAFF, 2010).

### 2.6.2 Rainfall

Mungbean requires a rainfall of about 400-500mm well distributed all through the growing period of the crop for its cultivation though this usually vary with location (AVRDC.2006). When it is planted at the late season it can lead to early flowering owing to low moisture coupled with high temperature and this will in turn reduce yield. When humidity is high due to excessive rainfall it predisposes the crop to diseases and harvest losses owing to delayed maturity (DAFF.2010).

### 2.6.3 Soil Requirement

Mungbean grows well on different soil types ranging from sandy soil, red laterite soils, a wide range of soils like red laterite soils and black cotton soil (AVRDC.2006). The best soil for mungbean cultivation is properly drained sandy loam soil that is fertile and with an average pH of about 6.75. Mungbean cannot thrive in saline soils and when planted in alkaline soils, they exhibit micronutrient deficiencies on their nodules and severe iron chlorosis (DAFF.2010).



## 2.7 Challenges of Mungbean Cultivation and Breeding Progress

Many disease-causing organisms pose great challenges to mungbean cultivation. Some of these pathogens include viruses, fungi, nematodes, bacteria. These pathogens cause several diseases like cercospora leaf spot, bacterial leaf spots, powdery mildew, mungbean yellow mosaic disease and bruchid. Several studies have

reported the action genes responsible for resistance to these pathogens cercospora leaf spot and bacterial leaf spot and bruchid are controlled by single dominant gene is controlled of resistance genes (Thakur *et al.,* 1978) while MYMD is controlled by a single dominant gene and complementary recessive gene (Poehlman, 1991). Young (1993) indicated that the resistance to mungbean powdery mildew is governed by multiple quantitative genes. Although biotic and abiotic stress are major limiting factors in the cultivation of many food crops Mungbean can easily adapt to tropical and subtropical low-lands and can relatively tolerate drought and heat stresses, but is greatly affected by soil salinity (Chankaew *et al.,* 2014). Salt stress has posed a great challenge in the cultivation of mungbean as it does not only affect the yield but also hinders the activities of Nitrogen fixing bacteria in the soil (Wati, 2017). Some Vigna species that are tolerant to salinity like the *Vigna marina* can be found growing around oceans under high saline condition but cannot be used for conventional breeding for salt tolerance as a result of their inability to be successfully crossed with mungbean and therefore cannot be used for breeding mungbean varieties that are tolerant to salinity (Chankaew *et al.,* 2014).Although some levels of salt tolerance has been found in mungbean germplasm, and may appear at different growth stages in different accessions, this makes the combination of these traits quite complex and breeding work difficult. Another major challenge limiting Mungbean cultivation is the yellow mosaic disease (MYMD). MYMD belongs to the begomoviral species which infect mungbean (Qazi *et al.,* 2007). The level of MYMD resistance present in the mungbean gene pool is not sufficient for generating resistant varieties and in a bid to overcome this, moderately resistant accessions and their hybrids were used for mutation breeding and this gave rise to many varieties that show high resistance against that disease (Ashraf *et al.,* 2001).The cross between mutants and a high yielding cultivar gave rise to the line known as NM94 which is now registered and used in various countries as a MYMD resistant line although susceptibility to MYMD-urd bean strain has been reported in some regions. Success has also been recorded in developing MYMD resistant lines (ML1628) in countries like Pakistan and India leading to the release of stable MYMD resistant lines which show high level of resistance to multiple strains of MYMD (Nair *et al.,* 2017). Bruchids (*Callosobruchus sp*.) are important pest of mungbean causing damage both in the field and during storage. The mechanism of infestation starts in the field where eggs are laid by the beetles on the pods. These eggs are hatched into larvae during storage and then mature into an adult, within the bean seed where they continue to lay their eggs. This pest is capable of

destroying all stored mungbean grains within the shortest possible time (Lambrides and Imrie 2000). The world vegetable center has discovered genetic resistance against this pest which was employed to develop resistant varieties in China, Korea and some other parts of the world (Yao *et al., 2015*).



### 2.8 Agro-ecological Diversity in Nigeria and Its Implications for Mungbean Adaptability

Nigeria boasts a rich tapestry of agro-ecological zones, each characterized by distinct climatic conditions, soil types, and cropping systems (Mponela *et al*., 2023). From the humid rainforests of the south to the arid savannas of the north, this diverse landscape presents both opportunities and challenges for agricultural production. Understanding the intricacies of Nigeria's agro-ecological diversity is paramount for assessing the adaptability of crops like Mungbean (Vigna radiata L. Wilczek) and optimizing their cultivation practices (Abraham *et al*., 2014). Abdullahi (2020) noted that Nigeria can be broadly divided into three main geographical regions: the southern rainforest zone, the central transitional zone, and the northern savanna zone. Each region exhibits unique climatic patterns and vegetation types, influencing agricultural suitability and crop distribution. Nigeria experiences a wide range of climatic conditions, ranging from tropical wet and dry climates in the south to semi-arid and arid climates in the north (Oguntunde *et al.,* 2017). Temperature, rainfall distribution, and length of growing seasons vary significantly across different regions, impacting crop growth, development, and yield potential. The soil diversity in Nigeria is equally remarkable, encompassing various soil types such as Ultisol, Afisols, Ferralsols, Nitosols, Luvisols, and Vertisols, among others (Tripathi *et al.,* 1992). Soil characteristics such as texture, pH, organic matter content, and nutrient availability vary across agro-ecological zones, influencing crop performance and nutrient management practices (Mulualem *et al.*, 2021). Traditional cropping systems in Nigeria are shaped by agro-ecological factors, cultural practices, and socioeconomic considerations. Ogazie *et al*., (2022) revealed that, from mixed cropping and shifting cultivation in the rainforests to mono-cropping and irrigation farming in the savannas, agricultural practices vary widely across regions, posing challenges and opportunities for crop diversification and integration. Bello et al., (2021) noted that the agro-ecological diversity of Nigeria presents both challenges and opportunities for Mungbean cultivation. Mungbean exhibits a remarkable degree of adaptability to diverse climatic conditions, making it suitable for cultivation across a wide range of agro-ecological zones in Nigeria. However, careful

selection of varieties and planting dates is essential to optimize performance and mitigate risks associated with extreme weather events (Waongo, 2015). Mungbean's ability to thrive in different soil types offers flexibility in cultivation practices. However, soil fertility management and nutrient supplementation may be necessary to address specific soil deficiencies and optimize yield potential, particularly in regions with poor soil quality (Bekunda *et al.,* 2010). Kumar *et al*., (2023) noted that Mungbean's relatively short growth cycle and nitrogen-fixing capabilities make it an ideal candidate for intercropping, crop rotation, and agroforestry systems. By integrating Mungbean into existing cropping systems, farmers can enhance soil fertility, reduce pest and disease pressure, and improve overall farm productivity.

## 2.9 Genetic variation and heritability estimates in Mungbean

### 2.9.1 Genetic Variation in Mungbean Genotypes

Genetic variation is the foundation of plant breeding. It provides the raw material for selecting and developing superior genotypes with improved yield, disease resistance, and adaptability (Somta *et al*., 2008). Genetic diversity ensures the adaptability of mungbean to different environmental conditions and stresses. It is crucial for developing resilient varieties that can withstand climate change (Kumar *et al*., 2018). Understanding and conserving genetic variation in mungbean is vital for maintaining the genetic resources necessary for future breeding efforts. This includes preserving wild relatives, landraces, and germplasm collections (Maxted *et al*., 2012).

The genetic base of cultivated mungbean is narrow, limiting the potential for genetic improvement. There is a need to explore and utilize the genetic diversity present in wild relatives and landraces (Tomooka *et al.,* 2011). Studies on the genetic diversity of mungbean in India have revealed significant variation among landraces and improved varieties. This diversity has been utilized to develop high-yielding and disease-resistant varieties (Ghafoor *et al.,* 2000; Paroda *et al*., 2005). Sangsiri *et al.,* (2005), In their research on the genetic variation of mungbean using molecular markers identified distinct genetic groups, aiding in the selection of parent lines for breeding programs. Another Genetic diversity studies by Chen *et al.,* (2008) focused on understanding the genetic structure of mungbean populations and identified markers associated

with important agronomic traits, this information can be used to enhance breeding efficiency. The integration of advanced genomic tools, such as genome-wide association studies (GWAS) and genomic selection, can accelerate the identification of beneficial alleles and the development of superior mungbean varieties (Nair *et al.,* 2019). SNP (Single Nucleotide Polymorphism) is a high-throughput and highly informative markers for genetic variation studies (Gupta *et al.,* 2013).



### 2.9.2 Heritability of trait in Mungbean

Heritability, the proportion of phenotypic variance attributable to genetic factors, is a critical parameter for understanding the genetic control of traits and predicting their response to selection. High heritability indicates that a trait is primarily governed by genetic factors and is less influenced by the environment, making it more amenable to improvement through breeding (Ravada *et al*., 2022). Conversely, low heritability suggests significant environmental influence, necessitating multi-environment trials or advanced genomic tools for effective selection. The genetic complexity of mungbean traits has been extensively studied using molecular tools such as genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping. A study by Kumar *et al*. (2023) evaluated 153 diverse mungbean genotypes across two environments for phenological traits (days to flowering and maturity) and agronomic traits (plant height, pod length, number of pods per plant, seeds per pod, and yield per plant). Broad-sense heritability estimates ranged from moderate to high (0.21–0.95), with traits like pod number per plant exhibiting high heritability (>0.90), indicating strong genetic control. This suggests that such traits can be effectively improved through phenotypic selection. Similarly, resequencing of 558 Chinese mungbean landraces identified 110 significant GWAS signals associated with nine agronomic traits. Candidate genes linked to these traits were involved in critical biological processes such as Brassinosteroid signalling, cell elongation, nutrient transport, and photosynthesis (Li *et al*., 2022). For instance, *Vradi04g07810*, a gene encoding serine carboxypeptidase located on chromosome 4, was associated with seed protein content (SPC) and seed starch content (SSC), demonstrating pleiotropic effects on seed quality traits. Phenotypic variability among mungbean genotypes reflects the combined effects of genetic and environmental factors. Studies have shown substantial variability for traits such as days to flowering, pod length, seeds per pod, and seed weight across different environments (Gayacharan *et al*., 2020). High

heritability estimates for yield-related traits such as pods per plant and harvest index suggest their suitability for direct selection in breeding programs. In contrast, low heritability for phenological traits like days to flowering highlights the need for multi-location trials to account for genotype × environment (G×E) interactions (Kumar *et al*., 2023). Environmental factors play a significant role in trait expression in mungbean. For instance, yield per plant exhibited high coefficients of variation across different environments due to drought stress and temperature fluctuations (Ravada *et al*., 2022). Phenological traits like days to flowering showed high heritability (>0.90) but variable mean values across locations due to differences in photoperiod sensitivity and climatic conditions.

### 2.10 Breeding efforts for improvement of Mungbean

Traditional breeding methods, such as selection and hybridization, have been extensively used to develop high-yielding and disease-resistant varieties in mungbean (Gupta *et al.* 2020). Early-maturing lines have been bred to fit multiple cropping systems, improving productivity. Additionally, researchers employ mutation breeding to introduce genetic variability and improve traits such as seed size and resistance to yellow mosaic virus (YMV), a major disease affecting mungbean production (Mishra *et al*. 2020). Modern approaches like molecular breeding and marker-assisted selection (MAS) have accelerated mungbean improvement by identifying genes responsible for desirable traits. Genomic tools and quantitative trait loci (QTL) mapping have been utilized to improve resistance to biotic and abiotic stresses. Moreover, biotechnology advancements, including genetic engineering and genome editing, hold promise for developing stress-resilient mungbean varieties (Vamshi *et al*. 2025). Continued breeding efforts aim to enhance climate resilience, improve nitrogen fixation, and increase nutritional value, making mungbean a more sustainable and valuable crop for global food security (Van Haeften *et al* 2023). Collaboration between research institutions and farmers ensures that improved varieties meet agricultural demands and consumer preferences.

#### 2.10.1 Conventional methods

Conventional breeding remains central to the genetic improvement of mungbean (*Vigna radiata* L. Wilczek), particularly for traits such as yield, earliness, disease resistance, and tolerance to abiotic stresses (Mogali and

Hegde, 2020). In mungbean improvement, hybridization and selection are essential components of the breeding process; however, they do not, by themselves, constitute breeding methods. Rather, they are integral steps applied within specific breeding methods designed to handle segregating populations and achieve genetic advancement.

The major conventional breeding methods used in mungbean include the bulk population method**,** pedigree method**,** single seed descent (SSD) method**,** and the backcross method (Khan *et al*., 2020; Das *et al*., 2018).

**Bulk Population Method:** This method involves advancing generations of hybrid populations in bulk without selection until the population reaches near homozygosity. Selection for desirable genotypes is deferred to later generations (typically $F_6$–$F_8$) when genetic segregation has stabilized. The method is suitable for traits governed by additive gene action and for crops like mungbean where large segregating populations can be maintained economically (Ayiecho and Nyabundi, 2025).

**Pedigree Method:** In this approach, selection begins in early generations, and the ancestry of each selected plant is recorded throughout the breeding process (Begna, 2021). This method allows precise tracking of superior genetic combinations and is particularly useful for improving complex traits such as yield and disease resistance in mungbean.

**Single Seed Descent (SSD) Method:** SSD emphasizes rapid generation advancement by growing one seed per plant from each generation until homozygosity is reached (Nair *et al*., 2019). This approach is efficient for shortening the breeding cycle, especially when combined with controlled environment facilities or off-season nurseries.

**Backcross Method:** The backcross method is used primarily for the introgression of specific desirable genes such as resistance to *mungbean yellow mosaic virus (MYMV)* or powdery mildew into an otherwise well-adapted variety (Sofi et al., 2021). Repeated backcrossing with the recurrent parent helps recover the original genotype while incorporating the target gene from the donor parent.

In all these methods, hybridization serves as the initial step to generate genetic variability, while selection is the driving force that identifies and retains superior recombinants. These processes, when strategically applied within the chosen method, enable the development of improved mungbean varieties that combine high yield potential with resilience to biotic and abiotic stresses. Although conventional methods are time-consuming and resource-intensive, they remain indispensable in mungbean breeding programs, particularly when integrated with modern genomic and molecular tools that accelerate selection efficiency and genetic gain (Tripathi *et al.*, 2020).

### 2.10.2 Association Mapping in Mungbean

Association mapping, also known as linkage disequilibrium (LD) mapping, is a genomic approach used to identify molecular markers linked with phenotypic traits in natural or breeding populations (Khan, 2013). It relies on detecting statistical associations between marker genotypes and phenotypic variations across a diverse set of genotypes that have undergone historical recombination events (Myles et al., 2009). Unlike traditional bi-parental quantitative trait loci (QTL) mapping, which requires the development of segregating populations, association mapping exploits existing natural diversity and therefore offers higher resolution and efficiency in detecting alleles underlying complex traits (Zhu *et al*., 2008).

The principle of association mapping is based on the non-random association of alleles at different loci (linkage disequilibrium), which decays with recombination over generations (Mackay and Powell, 2006). The extent of LD in a population determines the resolution and power of detection. Populations with slower LD decay require fewer markers but provide lower mapping resolution, while those with rapid LD decay, such as diverse germplasm panels, offer finer mapping resolution but require denser marker coverage (Kumar *et al.,* 2011). Association mapping has been extensively used in several crops, including rice, maize, soybean, and tomato, for identifying genomic regions controlling yield, disease resistance, and quality traits (Wang et al., 2008; Ranc *et al*., 2012; Wu *et al.,* 2014). In recent years, its application in mungbean (*Vigna radiata* L. Wilczek) has increased significantly due to the development of high-density molecular markers and the availability of reference genome sequences. In mungbean, association mapping and genome-wide association

studies (GWAS) have been successfully employed to dissect the genetic architecture of key agronomic and nutritional traits. For instance, Kang *et al*. (2014) identified single nucleotide polymorphisms (SNPs) significantly associated with seed size, pod length, and days to maturity using a mungbean diversity panel genotyped with SSR and SNP markers. Similarly, Ha *et al*. (2021) conducted GWAS on 293 mungbean accessions using 38,000 SNPs derived from DArTseq genotyping and identified loci associated with seed protein content, 100-seed weight, and yield components. Wu *et al.* (2020) also reported significant associations between SNP markers and seed mineral concentrations (Fe, Zn, and Mn), providing insights for biofortification breeding. Further, Suwannarat *et al*. (2022) applied GWAS to a diverse panel of mungbean genotypes and identified marker-trait associations related to drought tolerance and chlorophyll content under water stress conditions. These findings demonstrate that association mapping is a powerful tool for identifying quantitative trait loci (QTLs**)** and candidate genes contributing to trait variability in mungbean. Recent improvements in high-throughput genotyping platforms such as DArTseq, GBS (Genotyping-by-Sequencing), and SNP arrays have enhanced the resolution and accuracy of association mapping studies in mungbean. Integration of GWAS with transcriptomic and genomic selection approaches is now enabling the identification of functional alleles that can be used to accelerate molecular breeding and marker-assisted selection for yield, nutritional quality, and stress resilience (Kim *et al.,* 2021). Association mapping in mungbean has transitioned from low-resolution, marker-limited studies to genome-wide analyses supported by dense marker data and advanced statistical models. This progress has deepened understanding of the genetic basis of complex traits and holds great potential for developing improved mungbean cultivars adapted to diverse agro-ecological environments

### 2.10.3 Genome wide Association Studies in mungbean

To achieve breeding goals, it is essential to comprehend the genetics and genomes of phenological and yield-associated agronomic traits before introducing them to superior cultivars. Genome-wide association studies (GWAS) in mungbean have become an important field of research, focusing on understanding the genetic basis of various phenological and agronomic traits. Manjunatha *et al*. (2023) evaluated 153 diverse mungbean genotypes for traits such as days to heading, days to maturity, leaf nitrogen status, plant height, and yield per

plant. The research identified 50 SNPs significantly associated with these traits, explaining a considerable portion of the phenotypic variability. Also, Han *et al*. (2022) re-sequenced 558 Chinese mungbean landraces and identified 110 signals significantly associated with nine agronomic traits. A multi-GWAS study conducted by Pandey *et al.* (2024) revealed significant genomic regions for Mungbean yellow mosaic India virus resistance in urdbean (Vigna mungo (L.) across multiple environments, the study also identified genomic regions associated with resistance to the Mungbean yellow mosaic India virus. Genetic markers for MYMIV resistance in urdbean were uncovered, providing crucial insights for combating the disease and informing future breeding strategies. Genome-wise association study identified genomic regions associated with drought tolerance-related traits**,** the study re-sequenced 282 mungbean accessions to identify variants and performed GWAS to pinpoint genomic regions for drought tolerance (Chang *et al*., 2023). Sokolkova et al. (2020) utilizing the mini-core collection established by the World Vegetable Center, examined the genetic basis of variation in several important traits in mungbean.

Table 2.1 mung bean Putative QTLs identified through analyzing sequence similarity and conserved synteny between soybean and mung bean

| TRAIT | MUNGBEAN QTLs | SOYBEAN QTLs |
|---|---|---|
| First flower | 54 | 104 |
| Leaflet width | 55 | 61 |
| Leaflet length | 53 | 66 |
| Plant height | 171 | 268 |
| Pod maturity | 142 | 196 |
| Pod number | 40 | 59 |
| Seed oil | 178 | 236 |
| Seed oil to protein ratio | 0 | 16 |
| Seed protein | 140 | 356 |
| Seed weight | 245 | 272 |
| Seed weight per plant | 11 | 16 |

Source: Kim *et al*., (2015)

Although there are already genomic sequences available for mung beans, few QTL investigations have been carried out. Translational genomics has been used in a number of researches to describe the mung bean genome. Using genome-wide comparisons between Arabidopsis and Mung beans, flowering genes in Mung beans have been found (Kim *et al*. 2014). 207 genes in Arabidopsis are known to be involved in blooming; 129 of these genes are similar to genes found in mung beans. On a genomic map that was previously created for mung beans, several of these genes are situated close to SSR markers (Isemura *et al.* 2012). Furthermore,

Kim *et al.* (2014) discovered homology between five potential flowering-related genes in the mung bean and soybean by genome comparison.

**2.11 Effect of Genotype by Environment Interaction (GEI) in Mungbean**

G×E interactions play a critical role in mungbean breeding, influencing yield stability and adaptation. Understanding these interactions through advanced statistical methods enables breeders to develop robust and high-yielding mungbean varieties suitable for diverse environments (Adjei *et al*., 2022). The primary goal in mungbean breeding is to develop varieties with stable yield across different environments. Understanding G×E interactions help breeders identify genotypes that are specifically adapted to certain environments (specific adaptation) and those that perform well across a wide range of environments (general adaptation) (Yan & Kang, 2003). Varieties exhibiting high G×E interaction often show variable performance, which makes it challenging to predict their behavior in diverse conditions (Kang, 1998). Singh *et al.* (2015) evaluated mungbean genotypes across multiple environments in India and found significant G×E interactions affecting yield and other agronomic traits. They identified genotypes with stable performance and specific adaptation, which are crucial for targeted breeding programs. Similarly, Sharma *et al*. (2017) used the GGE biplot method to assess the performance of mungbean genotypes in different agro-ecological zones, their findings demonstrated the effectiveness of the GGE biplot in visualizing G×E interactions and guiding the selection of high-performing genotypes. Malik *et al*., 2011 and Sarwar *et al*., 2015 has also reported GxE interaction for traits like yield and yield parameters which suggesting the need to test genotypes in multiple environments. Other studies by Bangar *et al.,* 2019; Ratnasari *et al.,* 2020 in Southeast Asia have also reported significant G×E interactions for mungbean yield and other agronomic traits. These findings emphasize the importance of Multi-Environment Trials (METs) and the selection of stable genotypes for diverse agro-ecological zones.

Various statistical methods are employed to analyze G×E interactions, including AMMI (Additive Main Effects and Multiplicative Interaction), GGE (Genotype plus Genotype by Environment), and mixed models. These methods help in identifying stable genotypes and understanding the nature of G×E interactions (Ghafoor *et al.*, 2000; Asfaw *et al*., 2012). Many strategies have been adopted to manage GXE interactions in mungbean which includes selecting broadly adapted genotypes, developing region-specific varieties, and utilizing

genomic selection for predicting genotype performance across environments (Singh *et al.,* 2013; Tomar *et al.,* 2018). Integrating genomics into mungbean breeding can provide insights into the genetic basis of G×E interactions. Genomic selection and marker-assisted selection can accelerate the development of adaptable and high-performing mungbean varieties (Nair *et al*., 2019).



### 2.12 Adaptability Studies in Mungbean

Internationally, various studies have examined the adaptability of mungbean to diverse agro-ecological conditions, offering insights into its growth patterns, yield potential, and suitable management practices. For instance, research conducted in similar climatic regions of India, Bangladesh, and Southeast Asia has highlighted the significance of selecting appropriate varieties and cultivation techniques to maximize mungbean productivity in specific environments (Singh *et al*., 2016; Pandey *et al*., 2019).

However, extrapolating findings from these studies to the Nigerian context requires caution due to differences in soil types, climatic conditions, and farming practices. Hence, localized studies assessing the adaptability of mungbean to the various agro-ecological zones in Nigeria are crucial to provide region-specific recommendations for successful cultivation.

# CHAPTER THREE

## 3. Phenotypic Variation in morphological traits of Mungbean (Vigna radiata) Germplasm

### 3.1 Introduction

Mungbean (*Vigna radiata* L. wilczek), a vital legume crop, holds immense agricultural significance owing to its high nutritional value and adaptability to diverse environmental conditions (Bhardwaj, *et al.,* 2023). As global food demand rises, understanding the phenotypic variation in morphological traits within the Mungbean germplasm assumes paramount importance for sustainable crop improvement (Kgokong, 2022). Germplasm refers to the comprehensive assembly of genetic resources that encompass the diversity within a species, providing a rich reservoir for breeding programs and scientific exploration (Visioni *et al*., 2023). In the context of Mungbean, exploring the phenotypic variation in morphological traits across its germplasm becomes a pivotal avenue for unraveling valuable traits such as yield potential, resistance to diseases, and adaptability to changing climates (Dwived *et al*., 2017).

Morphological traits, play crucial roles in determining a crop's performance and adaptability (Khadka, *et al.,* 2016). The understanding of phenotypic variation in these traits within Mungbean germplasm offers insights into the genetic diversity, facilitating the selection of superior genotypes for breeding programme (Laskar, *et al*.,2023).

The objectives of the study were to:

a) explore and characterize the phenotypic variation in morphological traits across a diverse Mungbean collection

b) identify key traits contributing to the variability of the collection

c) determine the relatedness of accessions based on their similarities; and

d) determine the association among the traits scored

## 3.2 Materials and Methods

### 3.2.1Germplasm

Seeds of one hundred and twenty (120) genotypes of mungbean used for this experiment were introduced into Nigeria from the mini-core collection of the world vegetable center in 2021. The mini-core collection consists of mungbean accessions sourced from different parts of the world (Afghanistan, Australia, Brazil, France, India, Iran, Iraq, Korea Republic, Netherlands, Nigeria, Pakistan, Philippines, Thailand, and United States of America).

### 3.2.2 Phenotypic Characterization

This study was conducted at the teaching and research farm of Nnamdi Azikiwe University, Awka, south eastern Nigeria and at the teaching and research farm of Department of Crop Science, University of Uyo during two cropping seasons, 2022 and 2023. The site is located at latitude 6.24858° N, longitude 7.11545°E, with an elevation of 54 meters above sea level, and receives between 1,800-2500 mm of rainfall annually (Omoja *et al*., 2021). The experiment was laid in a 10 x 12 alpha lattice design with each block consisting of 10 genotypes. Genotypes were planted on beds with inter and intra-row spacing of 30cm × 20cm, respectively. Plots were labeled for data collection. The International Board of Plant Genetic Resources (IBPGR) has provided descriptors for mung beans, which was employed for the characterization of the 120 mungbean genotypes using morphological traits, the 17 characteristics were scaled before analysis. The following morphological traits were considered for the purpose of the study

### 3.2.3 Phenotypic data analysis

In order to determine the percentage of plant morphology depending on species and the geographic origin of the genotypes, descriptive analysis for qualitative data was carried out in R. For each of the fifteen geographic origins of the genotypes, the results were displayed as bar charts. Principal component analysis (PCA) was performed on all traits using the R "factoextra" and "vegan" packages.

Table 3.1: IBPGR Morphological Descriptors Used for Phenotypic Characterization of Mungbean genotypes



| S/N | Descriptors | Code | Description | Time of collection |
|---|---|---|---|---|
| 1 | Growth Habit | GH | 1-Erect, 2-Semi-erect, 3-Spreading | When the first pod changes colour |
| 2 | Growth Pattern | GP | 1-Indeterminate, 2-determinate | Recorded at 9 weeks after planting |
| 3 | Terminal leaflet shape | TLS | 1-deltoid, 2-ovate, 3-acute, 4-ovate lanceolate, 5- cuneate, 6-lobed, 7-other | Recorded at 9 weeks after planting |
| 4 | Terminal leaflet length | TLL | 1-Small (< 10cm), 2- Medium (10-13 cm) 3- Large (>13 cm) | Recorded at 9 weeks after planting |
| 5 | Leaf pubescence | LP | 1-Glabrous, 2-Pubescent | Recorded at 9 weeks after planting |
| 6 | Leaf colour | LC | 1-Light green, 2- Green, 3-Dark green, 4- others | At 50% flowering |
| 7 | Pod curvature | PC | 1-Least curve, 2-medium, 3-most curved | After harvest |
| 8 | Colour of petiole/leaf blade joint | CP | 1-Green, 2-purple, 3-Dark purple | Recorded at 9 weeks after planting |
| 9 | Colour of basal petiole | CB | 1-Green, 2-Purple,3-Dark purple | Recorded at 9 weeks after planting |
| 10 | Petiole length | PL | 1-Short (< 12 cm), 2-Medium (12-18 cm),3-Long (> 18 cm) | Recorded for the leaf at the fourth node |
| 11 | Raceme position | RP | 1-Mostly above canopy, 2-Intermediate<br>3-No pods visible above canopy | Recorded when the first pod changes colour |
| 12 | Calyx colour | CC | 1-Green,2-Greenish purple, 3-other | At 50% flowering |
| 13 | Colour of pod at immature stage | CPI | 1-Light green, 2-deep green, 3-other | Recorded at 9 weeks after planting |
| 14 | Seed colour | SC | 1-Yellow, 2-greenish, 3- light green<br>4-Dark green, 5-brown 6-mixed, 7- other | After harvest |
| 15 | Mottling on seeds | MOS | 1-Absent, 2-light, 3-medium, 4-heavy | After harvest |
| 16 | Lustre on seed surface | LSS | 1-Dull, 2-Shiny | After harvest |
| 17 | Seed shape | SS | 1-Round, 2-Oval, 3-Drum shaped, 4-Other | After harvest |

IBPGR (1985)

All traits underwent principal component analysis (PCA) with the use of the R "factoextra" and "vegan" packages (R Core Team, R: 2020). From the PCA result, eigenvalues and load coefficient values were extracted. A total of six primary components were chosen, each having an eigenvalue larger than one and a

cumulative value of 70.46%. Using the first two main components as specified by Peres-Nero *et al*., (2003) the contribution of each characteristic to the observed variability was calculated. Using the R "ggbiplot" tool, a biplot plot was produced using the first two principal components. Utilizing the Gower distance and a consistent data matrix, the "ape" software (Paradis & Schliep, 2019) was utilized to create pair-wise genetic similarity scores between genotypes for the purpose of cluster analysis. Using the FigTree program, the genetic distances were computed and utilized to create a hierarchical dendrogram using the "ward.D" clustering algorithm (Rambaut, A., 2010). In order to ascertain the phenotypic diversity within the collection, these methods were employed to examine variance patterns and genotype connections.

## 3.3 Results

### 3.3.1 Variability of qualitative morphological traits

The evaluation of 120 introduced mungbean (Vigna radiata L. Wilczek) genotypes revealed extensive variability in qualitative morphological traits, reflecting significant genetic diversity among the accessions. This diversity provides an essential foundation for selection and breeding towards improved adaptability and productivity under Nigerian agro-ecological conditions.

#### 3.3.1.1 Growth Habit and Growth Pattern

The mungbean genotypes exhibited predominantly erect (36.7%) and semi-erect (20.8%) growth habits, while a smaller proportion (14.2%) displayed a spreading habit. The predominance of erect and semi-erect types suggests suitability for high-density planting, mechanical harvesting, and better light interception. Erect plants also promote air circulation, reducing disease incidence, while spreading types though fewer may possess traits beneficial for ground coverage and weed suppression. For growth pattern, determinate genotypes (67.5%) were more frequent than indeterminate ones (32.5%). Determinate types are desirable for synchronized flowering and maturity, enabling single-time harvest and facilitating multi-cropping systems. Conversely, indeterminate genotypes, which flower and mature progressively, may be useful for extended reproductive phases and yield stability in stress-prone environments.

#### 3.3.1.2 Leaf Morphology and Photosynthetic Traits

Leaf-related traits exhibited notable variation. Ovate and ovate lanceolate leaf shapes were most common, accounting for nearly 49.2% of the genotypes. About 73.3% of the genotypes possessed small terminal leaflets, with only 1.7% having medium-sized leaves. Smaller leaves are advantageous in water-limited environments, as they minimize transpiration losses, while larger leaves may enhance photosynthetic surface area under well-watered conditions. Leaf pubescence was observed in 48.3% of the genotypes, compared to 20.8% glabrous types. Pubescent leaves confer drought tolerance and insect resistance by reducing leaf temperature and discouraging herbivory. Regarding leaf colour, light green leaves (53.3%) were most common, followed by green (35.8%) and dark green (10.8%). Light-green foliage reflects adaptation to high solar radiation and heat stress, while darker green leaves suggest higher chlorophyll concentration and photosynthetic capacity.

3.3.1.3 Pod and Floral Characteristics

Pod morphology displayed moderate variation. About 80% of the genotypes had medium-curved pods, while 20% were straight. Moderate curvature enhances threshing efficiency and resistance to shattering. Straight pods are advantageous in mechanical harvesting, reducing seed loss for pigmentation, 46.7% of the genotypes had green petioles, while 65% showed purple pigmentation at the basal petiole. The presence of anthocyanin pigmentation may contribute to UV protection and biotic stress tolerance. Petiole length was predominantly medium (45.8%) or short (43.3%), which favors compact canopy structure, enhancing light distribution and improving plant stability. Most genotypes (75%) produced racemes above the canopy, providing easy harvestability and better air circulation, thereby reducing fungal infection risks. Intermediate raceme positions (25%) may protect pods from excessive sunlight and mechanical injury. Calyx colour was largely greenish-purple (92.5%), a trait linked to anthocyanin accumulation, which is associated with stress adaptation. Immature pod colour also varied: light-green pods were predominant (69.2%) compared to deep-green pods (30.8%). Lighter pigmentation may correspond to early pod maturity, whereas darker pods tend to remain photosynthetically active for longer, possibly enhancing seed filling.

3.3.1.4 Seed Characteristics and Quality Traits

Seed-related traits showed significant variability, reflecting both adaptive and market-oriented diversity. Dark-green seeds were most prevalent (72.5%), followed by brown (15.8%) and light-green types (11.7%). Dark seeds are associated with better market preference, storage durability, and resistance to mechanical damage and fungal infection. Mottling on seeds occurred in varying degrees: heavily mottled seeds constituted 64.2%, lightly mottled seeds 24.2%, and 11.6% were plain. Seed coat mottling influences seed permeability and aesthetic value, both of which affect consumer acceptance. Lustre on the seed surface was mostly shiny (82.5%), indicating smooth seed coats that enhance resistance to fungal attack and improve visual appeal. Dull seeds (17.5%) may have thicker coats that delay imbibition but provide mechanical protection. Regarding seed shape, the majority (83%) were round, while the remainder included oval and drum-shaped forms. Round seeds are desirable for uniform cooking, better packing density, and mechanical grading efficiency, which are valuable attributes for commercial mungbean production and processing industries

**Table 3.2 Distribution of descriptor states describing the mungbean germplasm in percentage**

| State | GH | GP | TLS | TLL | LP | LC | PC | CP | CB | PL | RP | CC | CPI | SC | MOS | LSS | SS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.7 | 26.7 | 24.2 | 73.3 | 20.8 | 53.3 | 20.0 | 46.7 | 65.0 | 43.3 | 75.0 | 92.5 | 69.2 | 1.7 | 64.2 | 21.7 | 82.5 |
| 2 | 20.8 | 78.3 | 1.7 | 1.7 | 48.3 | 73.3 | 25.8 | 25.0 | 79.2 | 45.8 | 80.0 | 53.3 | 34.2 | 50.0 | 21.7 | 82.5 | 0.0 |
| 3 | 14.2 | 0.0 | 25.0 | 0.0 | 0.0 | 0.8 | 0.0 | 0.0 | 0.8 | 5.0 | 0.0 | 0.0 | 0.8 | 10.8 | 82.5 | 0.0 | 0.0 |
| 4 | 0.8 | 0.0 | 15.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 0.8 | 0.0 | 0.0 | 0.8 | 0.0 | 0.0 | 4.2 |
| 5 | 0.0 | 0.0 | 4.2 | 0.0 | 0.0 | 9.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 15.8 | 0.0 | 0.0 |
| 6 | 0.0 | 0.0 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 72.5 | 0.0 | 0.0 | 0.0 |
| 7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 0.0 | 0.0 | 0.0 |

*GH-Growth habit, GP-Growth pattern, TLS-Terminal Leaf shape, TLL-Terminal Leaf Length, LP-Leaf Pubescence, LC-Leaf Colour, PC- Pod Curvature, PC- Colour of Petiole, CB- Colour of Basal Petiole, PL- Petiole length, RP- Raceme Position, CC-Calyx Colour, CPI- Colour of Immature pods, SC- Seed colour, MOS, Mottling on seeds, Lustre on seeds, SS-Seed Shape

### 3.3.2 Relative contributions of traits to total variation

The phenotypic variance (%) shows the percentage of total variance explained by each principal component $PC_1$ explains 26.81% of the total variance, PC2 explains 11.56%, PC3 explains 10.35%, while PC4 explains

8.12%, PC5 7.27%, PC6 6.36% explains 4.45% of the total variance in the morphological characteristics (Table 3.2). The cumulative variance (%) shows how much of the total variance is explained by the cumulative set of principal components. In this case, the first two components (PC1 and PC2) together explain 38.36%, while PC1-PC4 explains 56.84% of total variance.

The eigenvalues represent the amount of variance explained by each principal component.

In this case, $PC_1$ has the highest eigenvalue (4.83), indicating that it explains the most variance in the data followed by PC2 (2.08) while the subsequent components contributed less to the overall variance as can be seen in Table 3.3. The loadings in each column represent the correlation between the morphological traits and the corresponding principal component. Colour of pod at immature stage (CPI) with absolute loading of 0.81 indicates that it contributed strongly to the PC1, this is followed by Colour of basal petiole CB (0.79) and Terminal leaflet shape TLS (0.68). For PC2 we can see that PL (0.73) contributed strongly followed by Terminal leaflet shape (0.47) in PC3 have Colour of basal petiole CB (0.73). The scores of each genotype on the first two principal components, PC1 and PC2 were plotted to assess the grouping of individual genotypes (Figure 3.1). Genotypes were coloured based on their geographical origin. The principal component analysis (PCA) biplot shows that the relationship between the measured traits and the mungbean genotypes based on their origin. PC1 on the x-axis accounted for 26.81% of the total variability, while PC2 explained 11.56 % of total variability and is shown on the y-axis. The different colors of the individuals (cultivars) represent the grouping of genotypes according to their sources. The contribution of traits to PC1 and PC2 is indicated by the length of the arrows. The longer arrows denote a higher contribution. Traits such as the colour of the pod at the immature stage (CPI), the colour of the basal petiole (CB) and Terminal leaflet shape have high loadings on PC1(0.81, 0.79 and 0.68), indicating they are major contributors to the variance explained by PC1.Traits such as petiole length (PL) and Terminal leaf shape (TLS) have high loadings (0.73 and 0.47) on PC2 suggesting significant contributions to the variance explained by PC2.

**Table 3.3 Contribution of individual traits to phenotypic variation**

| Variable | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| GH | -0.32 | 0.05 | -0.06 | 0.38 | 0.68 | 0.06 |
| GP | 0.65 | -0.06 | 0.23 | 0.20 | -0.02 | -0.20 |
| TLS | 0.68 | 0.43 | 0.15 | -0.12 | -0.14 | -0.13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| TLL | -0.31 | 0.46 | 0.15 | 0.55 | -0.34 | -0.14 |
| LP | 0.45 | 0.22 | 0.21 | 0.27 | 0.45 | -0.42 |
| LC | 0.54 | -0.04 | -0.09 | 0.34 | -0.19 | 0.47 |
| PC | -0.20 | 0.73 | 0.28 | -0.19 | -0.21 | -0.15 |
| CP | -0.67 | 0.34 | 0.20 | 0.10 | -0.25 | 0.01 |
| CB | 0.79 | 0.00 | -0.06 | 0.27 | 0.06 | 0.19 |
| PL | 0.56 | 0.39 | -0.02 | 0.17 | 0.10 | 0.32 |
| RP | -0.67 | 0.33 | 0.10 | 0.13 | 0.30 | 0.06 |
| CC | -0.53 | 0.28 | 0.01 | 0.17 | -0.35 | 0.29 |
| CPI | 0.81 | 0.43 | 0.00 | -0.21 | -0.07 | -0.03 |
| SC | -0.23 | -0.05 | 0.73 | 0.12 | -0.07 | -0.31 |
| MOS | 0.12 | -0.39 | 0.66 | -0.20 | 0.15 | 0.31 |
| LSS | -0.03 | 0.20 | -0.59 | 0.49 | -0.20 | -0.35 |
| SS | 0.18 | 0.21 | 0.52 | 0.49 | -0.11 | 0.19 |
| Eigenvalue | 4.83 | 2.08 | 1.86 | 1.46 | 1.31 | 1.14 |
| Variance (%) | 26.81 | 11.56 | 10.35 | 8.12 | 7.27 | 6.36 |
| cumulative variance (%) | 26.81 | 38.36 | 48.72 | 56.84 | 64.11 | 70.46 |

### 3.3.3 Correlation among studied traits

The correlation matrix of the 17 observed morphological traits of mungbean are displayed in Figure 3.3, to enable us to explore the associations between them. The blues colour circles represent a positive association while the orange circle represents a negative association. A greater coefficient is reflected by a colour of higher intensity. The relationships between various phenotypic traits of the mungbean genotypes was illustrates using the correlation coefficient. Each cell in the table shows the correlation coefficient between two traits.

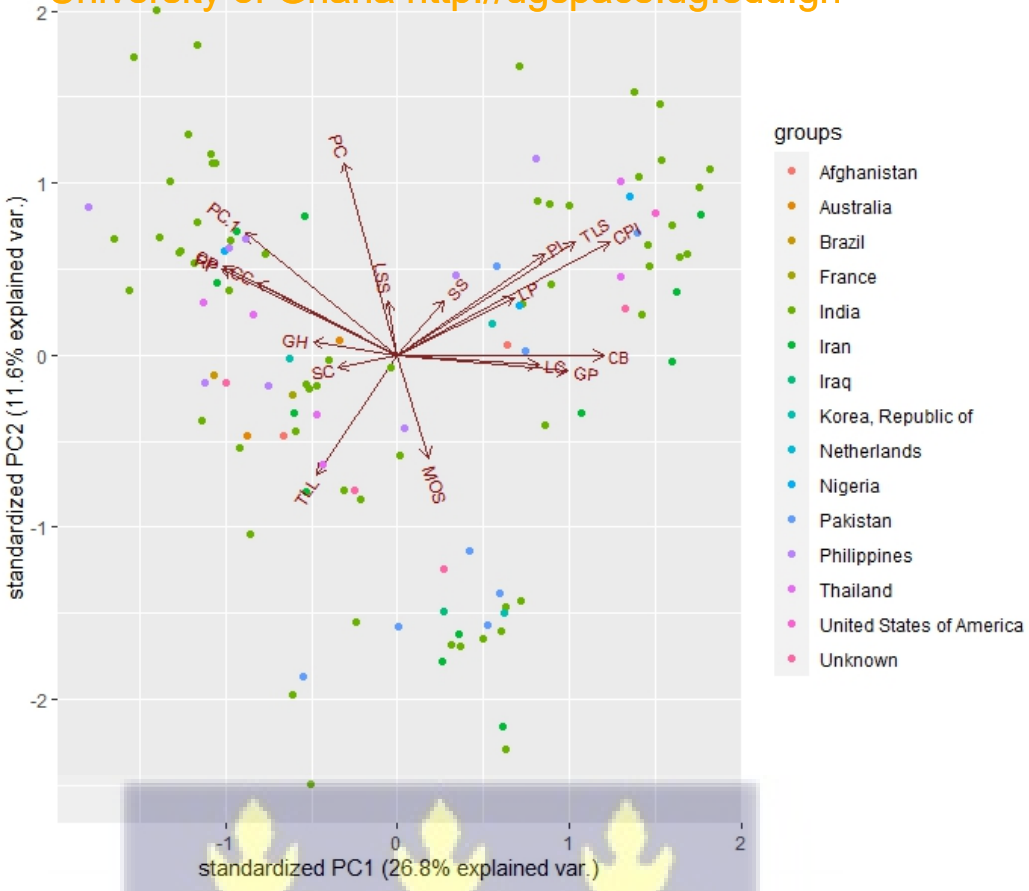


Figure 3.1 The two-dimensional plot of the first two principal components (PC1 and PC2)

indicating how changes in one trait are associated with changes in another. A Strong Positive Correlations was observed between Colour of pod at immature stage (CPI) and Colour of basal petiole (CB) (0.81).

This suggests that as the colour intensity of the pod at the immature stage increases, the colour of the basal petiole also becomes more intense. The Terminal leaflet shape (TLS) and Growth habit (GH) also showed positive correlation (0.68) which indicates that variations in terminal leaflet shape are associated with changes in the growth habit of the plant. Moderate positive correlation was observed between Petiole length (PL) and Terminal leaflet shape (TLS) (0.56), this was also followed by Leaf pubescence (LP) and Leaf colour (LC) (correlation coefficient: 0.45). A few Negative Correlations were observed between Raceme position (RP) and Colour of petiole/leaf blade (CP) (-0.67). The same was also observed for Growth habit (GH) and Colour of pod at immature stage (CPI) (-0.67) suggests that as the growth habit becomes more pronounced, the colour of the pod at the immature stage tends to decrease.

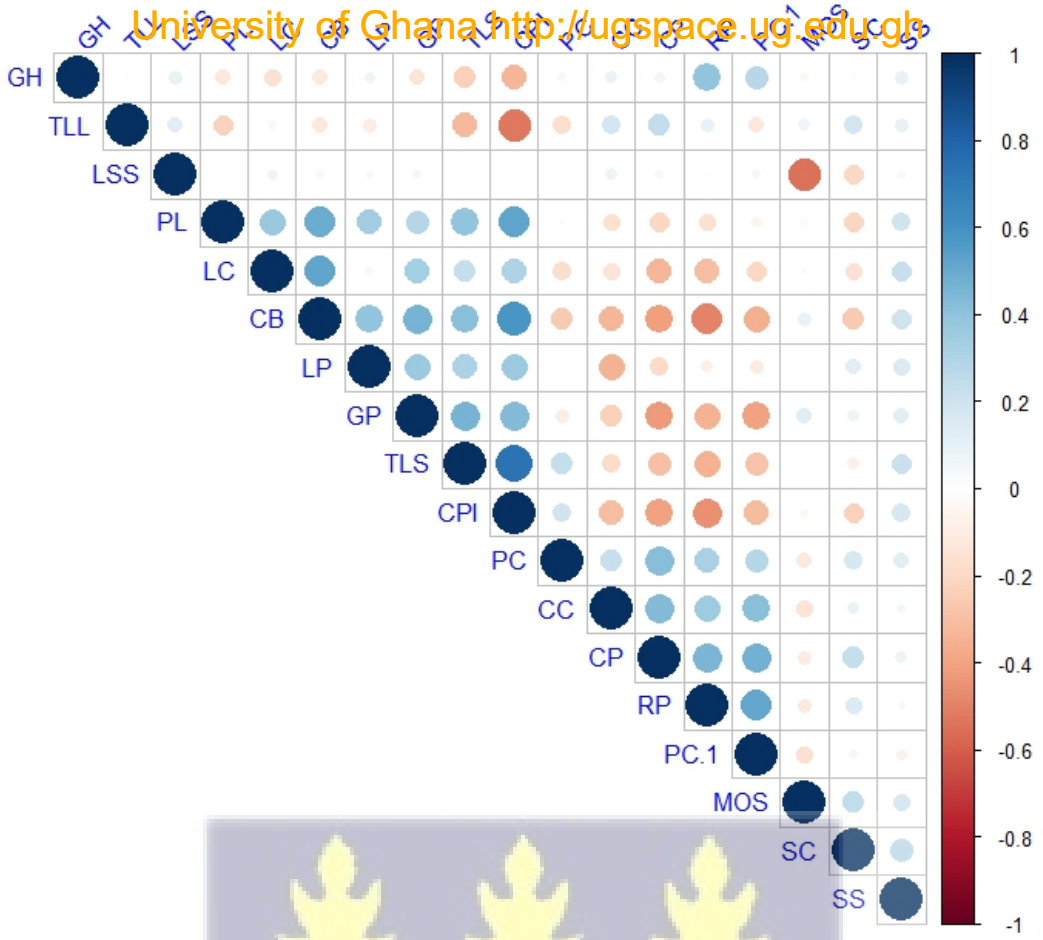


Fig. 3.2 Correlation of 17 morphological traits of mungbean

### 3.3.4 Genetic relatedness among mungbean genotypes

The dendrogram (Figure 3.3) shows two distinct clusters (A and B), representing groups of genotypes with similar morphological characteristics. Each major cluster was further divided into sub-clusters, reflecting varying degrees of genetic similarity among accessions. This clustering pattern reveals considerable genetic diversity within the mungbean germplasm collection.

The presence of closely grouped genotypes suggests possible duplicates, which could be streamlined to avoid redundancy in the germplasm collection. Conversely, the wide separation among certain accessions indicates genetically divergent lines that can serve as valuable parents in future breeding programs aimed at broadening the genetic base of mungbean in Nigeria. Identifying and maintaining genetically distinct accessions is crucial for effective germplasm conservation and the formulation of a core collection representing maximum diversity with minimum redundancy.

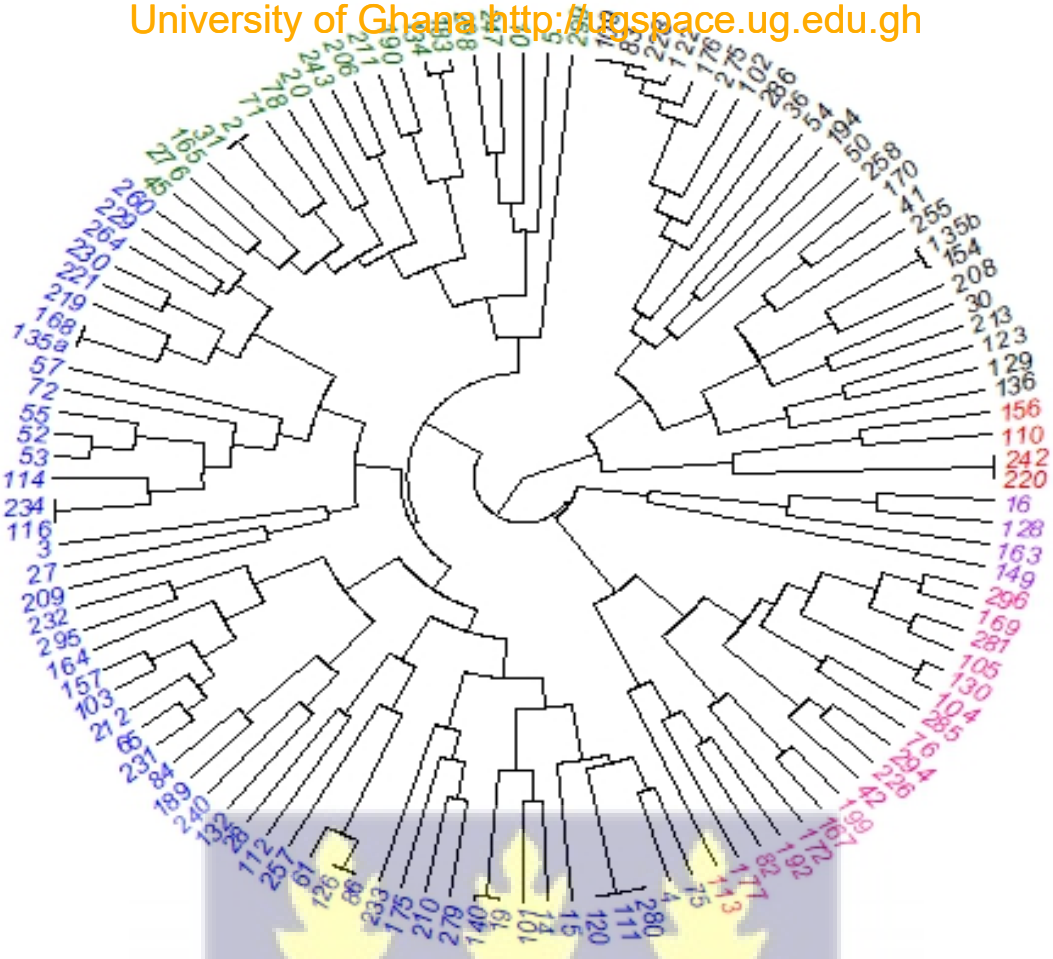


Fig.3.3: Diversity among 120 mungbean genotypes based on phenotypic traits

### 3.4 Discussion

It is well recognized that morphological traits, have a significant impact on the evolution of crop varieties (Gayacharan et al 2020). A seed's shape, color, and luster are crucial characteristics that influence consumer selection. As a result, regional and local tastes are what drive mungbean breeding programs. For instance, in the eastern states of India, little seed mungbean is highly valued and cost higher than bold seed mungbean. Likewise, bean sprouting companies prefer cultivars with green hypocotyls over those with purple ones (Yimram, et al., 2009). Genotypes with bright green seed coats are typically favoured over those with drab coats. Mature pods with a black color help to lessen the yellowing of the seeds inside the pod. The length and density of trichomes influence the inclination of specific species of insect pests (Hasanuzzaman, et al., 2016; Sagar, et al., 2017; Taggar & Gill, 2012). The bruchid (Callosobruchus species) infestation is also influenced by the texture of the seed surface (War et al., 2017). Genotypes with twinning growth habit are appropriate

for intercropping, which is still practiced in subsistence agricultural systems, with crops including pigeon pea, millets, and sorghum. Morphological characterization is a highly important method for defining germplasm of crop species and provides meaningful criteria for selecting materials with desirable traits for crop improvement activities (Tripathi *et al*.,2022; Adjei et al., 2022). This study successfully used 17 morphological characteristics to measure variability in the Mungbean genetic resource, effectively distinguishing genotypes based on their relationships or differences. Having a better understanding of the existing mungbean germplasm in Nigeria is crucial for breeding new genotypes with new or improved characteristics (Obasi *et al.*, 2024). Altogether, the 17 traits significantly contributed to phenotypic variability, demonstrating a high degree of morphological variation within the mungbean genotype used in the current study. Similar observation was reported by Desai and Modi (2023) for 17 morphological traits among 26 mungbean genotypes. The first six principal component contributed over 70% of the total variation. this agrees with the work of Shyamalee, *et al* (2016) who reported similar value for twelve agro-morphological traits of mungbean, Paliwal *et al*. (2022) reported that PC1 displayed a maximum variability of 18.139% and an eigen value of 2.177. In their evaluation of 20 components, Jadhav *et al*. (2021) found that components PC1 through PC7 which are obtained from the original data and have eigen values greater than one accounted for 70% of the variation. By evaluating 17 quantitative characteristics of mung beans, Yoseph *et al*. (2021) discovered that 7 principal components, with PC1 having an eigen value of 4.246, accounted for 80.1% of the variation. The first two principal components, or PC1 and PC2, respectively, showed 36.74 and 21.12% of the total variation, according to Desta *et al.* (2023). Sharma et al. (2023) found that eight PCs altogether, contributed 78.8% of the total variance. Nalajala *et al*. (2023) from his evaluation of twenty-eight quantitative and qualitative traits of mungbean genotypes also reported 71.22% explained by the first nine PCs.

The biplot using PC1 and PC2 explain 38.6% of the total variance, highlighting that these two components provide substantial but not exhaustive insight into the data structure. Identifying clusters of mungbean genotypes with desirable traits can inform selection strategies (Azam, *et al*., 2023). Manjunatha et al., (2023) opined that the presence of mixed colors within clusters suggests a high level of phenotypic diversity across origins, which could be advantageous for breeding programs aiming to combine traits from different origins. The plot shows the distribution of genotypes in the space defined by PC1 and PC2, with colors indicating their

origin. If genotypes from the same origin form tight clusters, it suggests they share similar phenotypic traits. If colors are mixed within clusters, it indicates phenotypic traits are not strongly tied to the origin



The strong positive correlations observed between Colour of pod at immature stage and Colour of basal petiole (0.81) aligns with the findings of Kurrey, (2023) for soybean accessions in India. This could imply that as the colour intensity of the pod at the immature stage increases, the colour of the basal petiole also becomes more intense. This relationship is important for selecting genotypes with specific colour characteristics for breeding programs (Azam *et al.,* 2023). The Terminal leaflet shape (TLS) and Growth habit (GH) also showed positive correlation (0.68) indicating that variations in terminal leaflet shape are associated with changes in the growth habit of the plant (Ibrahima, 2012). This could be useful for morphological classification and breeding purposes. Moderate positive correlation was observed between Petiole length (PL) and Terminal leaflet shape (0.56) which suggests that as the petiole length increases, there is a corresponding change in the terminal leaflet shape. This relationship can be explored further for breeding genotypes with desirable leaf traits this was also followed by Leaf pubescence (LP) and Leaf colour (LC) (correlation coefficient: 0.45), indicating that genotypes with more leaf pubescence tend to have a specific leaf colour. This can be significant for selecting traits related to leaf morphology and plant protection. A few Negative Correlations were observed between Raceme position (RP) and Colour of petiole/leaf blade (CP) (-0.67), indicating that as the raceme position changes, there is an inverse change in the colour of the petiole/leaf blade. This relationship can be crucial for understanding how different morphological traits interact and affect each other. The same was also observed for Growth habit (GH) and Colour of pod at immature stage (CPI) (-0.67) suggests that as the growth habit becomes more pronounced, the colour of the pod at the immature stage tends to decrease. This information can be used in selecting for specific growth habits and pod colour characteristics. Ganguly et al. (2012) also reported that most of the measured morphological traits were not positively correlated with each other while some others showed a positive correlation. These correlations provide valuable insights for plant breeders aiming to improve specific traits in mungbean genotypes. By understanding of these relationships, breeders can make informed decisions when selecting parent plants for cross-breeding to enhance desired traits such as pod colour, leaf morphology, and growth habits. The strong correlations can also aid in developing new mungbean varieties that are better adapted to specific environmental conditions, enhancing

their overall performance and yield. (Paradis *et al*, 2019). By emphasizing genotypes that are genetically similar or different, the clustering was used to highlight the genetic variety within the mungbean germplasm collection. The ability to distinguish distinct genotypes with desired traits, which can be employed in breeding programs to improve crop performance and adaptability is made possible by this knowledge. (Cobb, *et al*.,2013). The tight grouping of certain accessions within sub-clusters suggests possible duplicates or closely related lines, pointing to the need for rationalization to reduce redundancy in the germplasm collection (Schafleitner *et al.*, 2015). Conversely, the wide separation between genotypes such as 130 (Cluster A) and 55 (Cluster B) indicates significant genetic divergence, which is highly desirable for broadening the genetic base and generating heterotic combinations in breeding programmes (Somta *et al*., 2022). From a conservation perspective, the clustering provides a sound basis for establishing a core collection of representative accessions from each major group, enabling efficient management and sustainable utilization of mungbean genetic resources (Schafleitner *et al.,* 2015; Somta *et al.,* 2022). Overall, the dendrogram analysis offers actionable guidance for both breeding (to select diverse parents for yield and adaptation improvement) and germplasm conservation, to ensure long-term genetic resource security in Nigeria

### 3.5 Conclusion



This study explored the phenotypic variation in 120 mungbean (Vigna radiata) genotypes from 15 geographical regions, providing crucial insights into genetic diversity for breeding programs. Significant variability was observed in key morphological traits, with colour of pod at the immature stage (CPI), colour of basal petiole (CB), and terminal leaflet shape (TLS) emerging as major contributors to phenotypic variation. Principal Component Analysis (PCA) showed that the first six components explained over 70% of the total variance, highlighting these traits as valuable selection markers. Strong trait correlations suggested opportunities for trait-linked selection, while hierarchical clustering grouped the genotypes into three distinct clusters with several sub-clusters, emphasizing genetic diversity that can be leveraged for hybridization. This study reaffirms the importance of morphological characterization in mungbean improvement, offering a solid foundation for future breeding programs. Further research should integrate molecular marker analysis to enhance precision in genotype selection and accelerate crop improvement for food security

# CHAPTER FOUR

## 4.0 Genetic variation among Mungbean breeding lines based on DArTseq Single Nucleotide Polymorphic Markers

### 4.1 Introduction

The enhancement of desirable traits, such as yield, resistance to diseases, and tolerance to environmental stresses, is crucial for ensuring sustainable production (Bigini *et al*., 2021). Understanding the genetic variation among Mungbean breeding lines is a fundamental step towards achieving these goals. Advancements in molecular biology techniques have revolutionized the study of genetic diversity. Among these, Diversity Arrays Technology using next-generation sequencing (DArTseq) has emerged as a powerful tool for high-throughput genotyping; enabling the identification of Single Nucleotide Polymorphic (SNP) markers (Thomson. 2014) SNPs are valuable genetic markers due to their abundance and widespread distribution throughout the genome, providing insights into the genetic architecture of traits.

The genetic diversity among Mungbean breeding lines can be explored through the application of DArTseq-based SNP markers. This approach allows for a comprehensive analysis of the allelic variations present within the breeding lines, shedding light on the underlying genetic factors that contribute to desirable traits. Several studies have highlighted the efficacy of DArTseq in assessing genetic diversity in various crops, including legumes. For instance, Edema *et al.,* 2023 utilized DArTseq to characterize genetic variation in cowpea breeding lines, revealing valuable insights into the population structure and allelic diversity. The genetic diversity and population structure of Uganda's yam were also assessed by Amponsah *et al*., 2023 using the same technology. In this context, our study aims to investigate the genetic variation among Mungbean genotypes using DArTseq-derived SNP markers. By employing this high-throughput genotyping approach, we aim to unravel the intricacies of the Mung Bean genome, identify key genetic markers associated with desirable traits, and contribute to the ongoing efforts in Mung Bean breeding programs. Therefore, the objective of this study was to assess the genetic diversity and population structure of mungbean (*Vigna radiata*) genotypes using DArTseq-derived SNP markers.

## 4.2 Materials and methods



### 4.2.1 Genetic materials

A total of two hundred and ninety-three genotypes of *Vigna radiata*, originating from (Afghanistan, Australia, Brazil, France, India, Iran, Iraq, Korea Republic, Netherlands, Nigeria, Pakistan, Philippines, Thailand, and United States of America) were sampled (Appendix 1). The bulk of the genotypes were part of the world vegetable center mini-core collections. The genotypes were planted in an alpha lattice design

### 4.2.2 Leaf sampling for genotyping

A technique outlined in KBS-9370-001 was used to gather mungbean leaf samples in order to extract DNA from them. Five weeks after planting, leaf samples from three (3) tagged plants were punched out using a leaf puncher and placed onto each of the 96-well plates. After being oven-dried at 80 °C, the leaf samples were shipped to DArT P/L, Australia, for genotyping.

### 4.2.3 DNA extraction

Genomic DNA was isolated from the Mungbean leaf samples using the QIAGEN DNeasy plant micro kit, following the supplier's instructions (Qiagen, Valencia, CA, USA). DNA was quantified using a Qubit fluorometer, and samples exceeding 100 ng/µl were stored for quality control. Using 25 ng and 50 ng lambda DNA markers, electrophoresis on 0.8% agarose gels was used to assess the integrity of the DNA. Spectrophotometry was used to measure the quality of the DNA at 230, 260, and 280 nm. Samples containing intact DNA, an absorption ratio of 260/280 nm of approximately 1.8, and a concentration more than 100 ng/µl were used for genotyping by sequencing (GBS).

### 4.2.4 SNP discovery by DArTseq™ technology

DArTseq is a genotyping-by-sequencing technique designed to aid in marker identification by sequencing the most informative genomic DNA sample representations. It uses Next-Generation Sequencing technologies to achieve this. GBS was carried out by Diversity Arrays Technology (DArT P/L, Australia). 24,870 SNPs in all were obtained.

### 4.2.5 Genetic analysis

#### 4.2.5.1 Data filtering



SNPs with missing chromosome locations were eliminated, and duplicate and monomorphic loci resulting from the elimination of certain genotypes were filtered using Trait Analysis by aSSociation, Evolution, and Linkage (TASSEL 5.2.31) software (Bradbury *et al.* 2007). A significant number of SNPs were left behind after filtering, and they were mapped to the chromosomes of mungbean (Kang *et al*. 2014). The minimum allele frequency (MAF) was set at 0.05 in order to eliminate low frequency SNPs. And the study was conducted using 5,037 SNPs that were kept. R2 values ≤0.1 were used to choose markers.

#### 4.2.5.2 Analysis of genetic diversity

Using PowerMarker version 3.25 (Liu and Muse 2005), the polymorphic information content (PIC), gene diversity, and allele frequency were determined for every marker. The PIC value is defined as each marker's relative value to the amount of polymorphism revealed (Lu *et al*. 2009). Gene diversity is the likelihood that two randomly chosen alleles from the test sample differ; heterozygosity, or the percentage of heterozygous loci found in each inbred line, was also determined (Lu *et al.* 2009). Roger's genetic distance (Rogers 1972) was used to determine the genetic distance between the lines. Using Power Marker version 3.25, the neighbour joining approach was used to create a dendrogram using the genetic distance matrix (Liu and Muse 2005). The resulting trees were visualized using MEGA version 11 (Tamura *et al.* 2011).

#### 4.2.5.3 Population structure

In all, 5037 SNPs were selected and utilized for the investigation. PowerMarker (v3.25) was used to estimate the polymorphism information content (PIC), and TASSEL (v5.2.52) was used to assess minor allele frequencies and SNP marker information (Liu and Muse 2005). The observed and predicted heterozygosity were computed using R's "adegenet" package (R Core Team 2020). The STRUCTURE program (v2.3.4), which employs clustering based on a Bayesian model (Evanno *et al*. 2005; Porras-Hurtado *et al*. 2013), was used to estimate the number of hypothetical subpopulations (K). An admixture model based on the Hardy-Weinberg equilibrium and associated allele frequencies was used in the STRUCTURE analysis, with a burn-in period of 10,000 Markov-chain Monte Carlo iterations.

Ten separate runs were conducted for each of the ten population numbers (K), which varied from 1 to 10. The structure outputs were further evaluated using STRUCTURE HARVESTER (Earl and vonHoldt 2012), which allowed for the unique peak in the change of likelihood (K) to be identified as the ideal K value.



## 4.3. **Results**

### 4.3.1 Functional Characterization, Genetic Distances and Relationships of DArTseq-SNPs on Mungbean chromosome

The DArTseq genotyping of 299 Mungbean genotypes produced 24,870 SNPs in total. 5,037 high-quality SNPs (20.25%) spread across *Vigna radiata's* 11 chromosomes (Fig. 4.1 and Table 1). According to marker density across chromosomes, chromosome 1 had the most SNPs (689 SNPs; 13.68%), followed by chromosomes 8 (649 SNPs; 12.88%) and 6 (581 SNPs; 11.53%), while chromosome 10 had the fewest SNPs (287 SNPs; 5.70%) (Fig 4.1 and Table 4.1). The average PIC value for all the markers was 0.27, with a range of 0.25 to 0.29 for quality metrics. The expected heterozygosity ranged from 0.31 to 0.37, with a mean of 0.33. On the other hand, the detected heterozygosity had an average of 0.13 and varied from 0.04 to 0.24. In a similar vein, Table 4.1 indicates that the minor allele frequency (MAF) averaged 0.24 and varied from 0.23 to 0.27. A heat map of the genetic links between several mungbean genotypes shows that lighter colours indicate more distant linkages while deeper colours indicate closer ones. It is essential to fully understand genetic diversity in order to select genotypes for breeding operations.

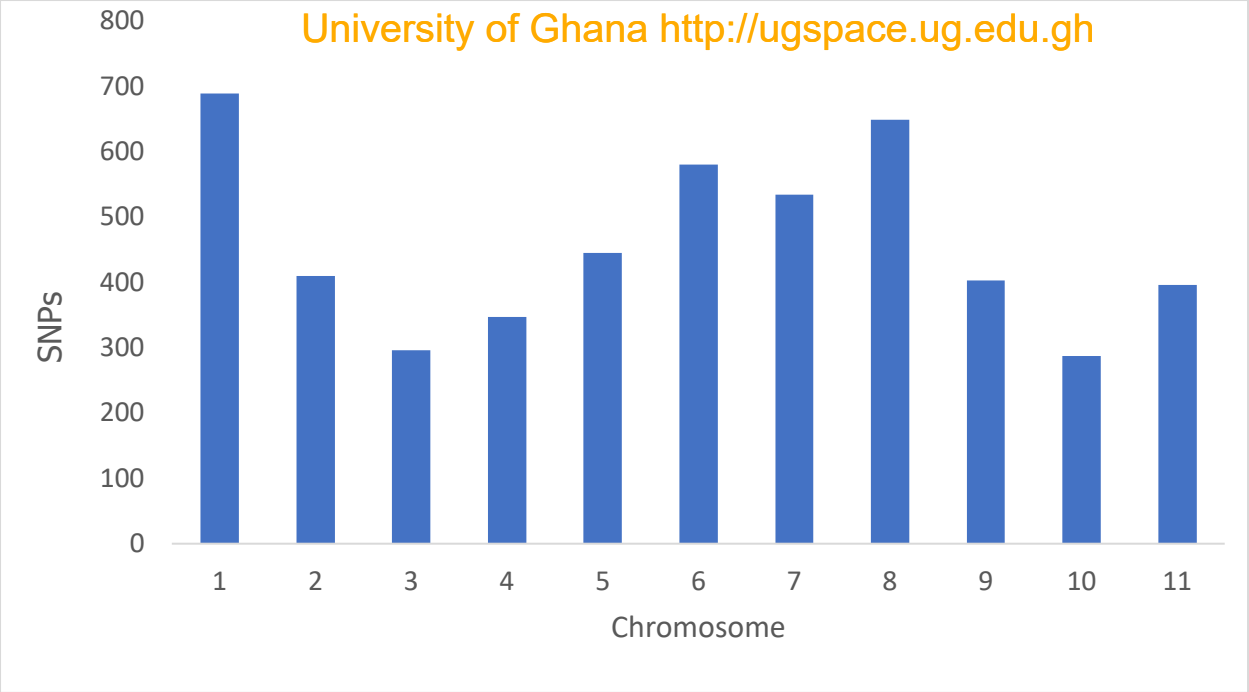


Fig.4.1: Number of SNP markers distributed across the 11 Chromosomes

Table4.1 Quality and summary statistics of DArTseq-SNPs on Mungbean chromosomes

| Chr | SNPS | GD | PIC | MAF | Ho | He |
|---|---|---|---|---|---|---|
| 1 | 689 | 0.33 | 0.27 | 0.24 | 0.05 | 0.34 |
| 2 | 410 | 0.33 | 0.27 | 0.24 | 0.10 | 0.32 |
| 3 | 296 | 0.36 | 0.29 | 0.27 | 0.04 | 0.36 |
| 4 | 347 | 0.34 | 0.27 | 0.25 | 0.23 | 0.33 |
| 5 | 445 | 0.33 | 0.27 | 0.24 | 0.12 | 0.33 |
| 6 | 581 | 0.33 | 0.27 | 0.25 | 0.11 | 0.35 |
| 7 | 534 | 0.32 | 0.26 | 0.23 | 0.10 | 0.31 |
| 8 | 649 | 0.32 | 0.26 | 0.23 | 0.24 | 0.31 |
| 9 | 403 | 0.35 | 0.28 | 0.27 | 0.12 | 0.37 |
| 10 | 287 | 0.32 | 0.26 | 0.23 | 0.15 | 0.32 |
| 11 | 396 | 0.31 | 0.25 | 0.23 | 0.22 | 0.32 |
| Total/Average | 5037 | 0.33 | 0.27 | 0.24 | 0.13 | 0.33 |

*Chr: Chromosomes, MAF: Minor allele frequency, GD: Gene diversity, PIC: Polymorphic information content, Ho: Observed heterozygosity, He: Expected heterozygosity

### 4.3.2 Interrelationships among genotypes



Two Primary clusters (A and B) were discovered by the cluster analysis (Figure 4.4), and two sub- clusters B1 and B2 were also found. Significant genetic variation between genotypes is indicated by these groups. Three genotypes (127,118 and 292) make up Cluster A, where as additional sub-cluster and sub-sub clusters in cluster B gave more intricate genetic linkages that may be helpful in for breeding and conservation purpose

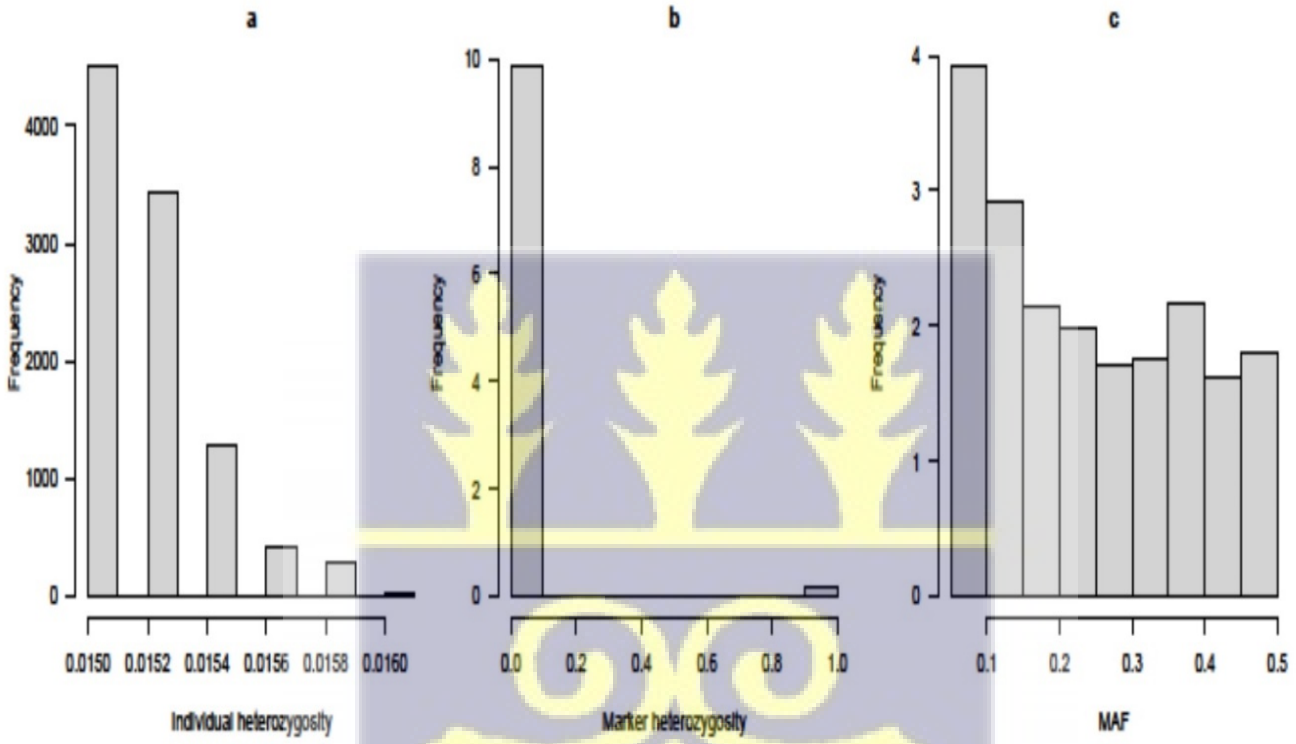


Fig 4.2. Frequency of heterozygous genotypes and heterozygosity of 5,037 SNP markers generated using DArTseq platform across 293 Mungbean genotypes

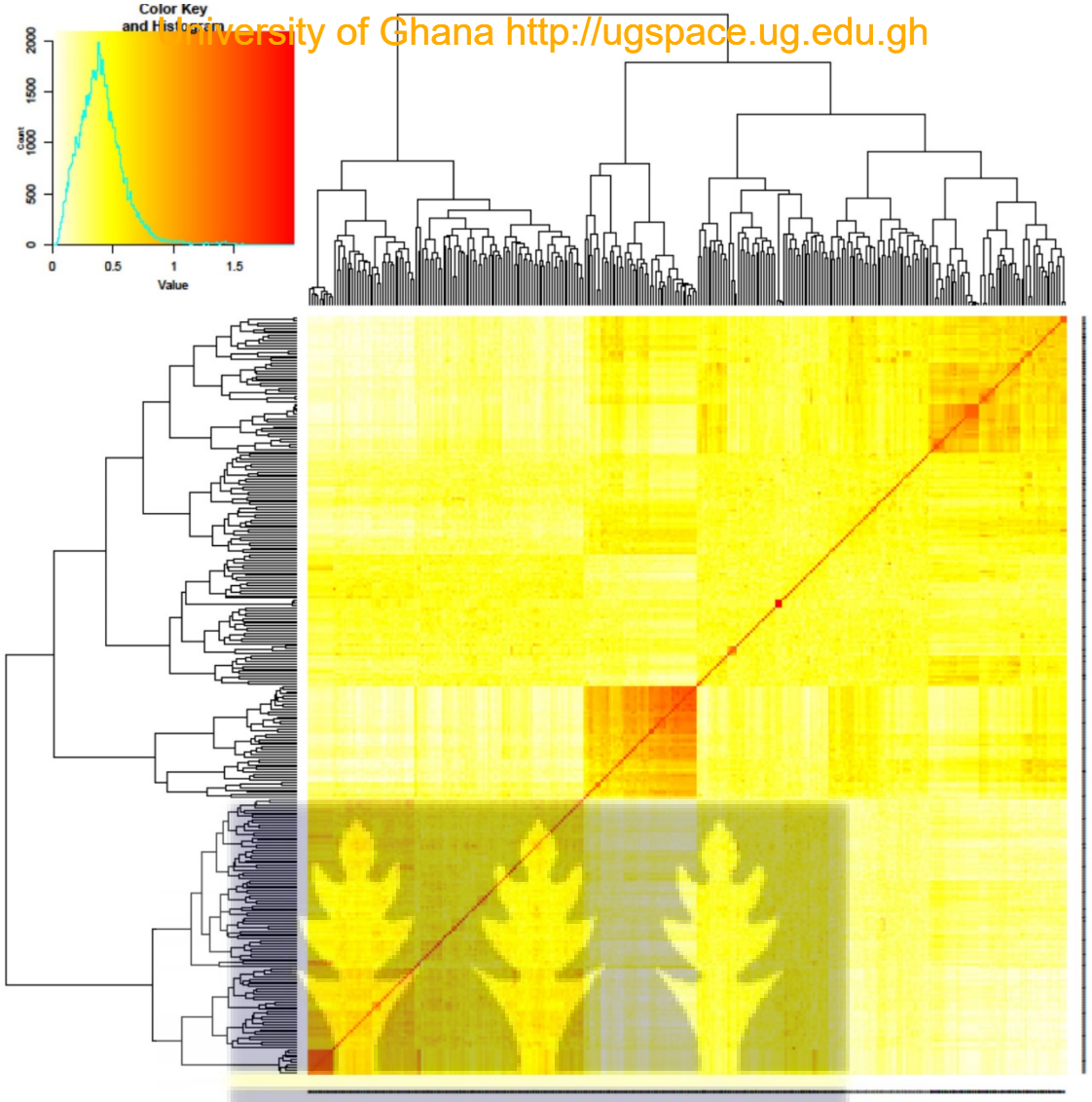


Fig 4.3. Heat map plot of kinship matrix using average linkage clustering based on SNP markers

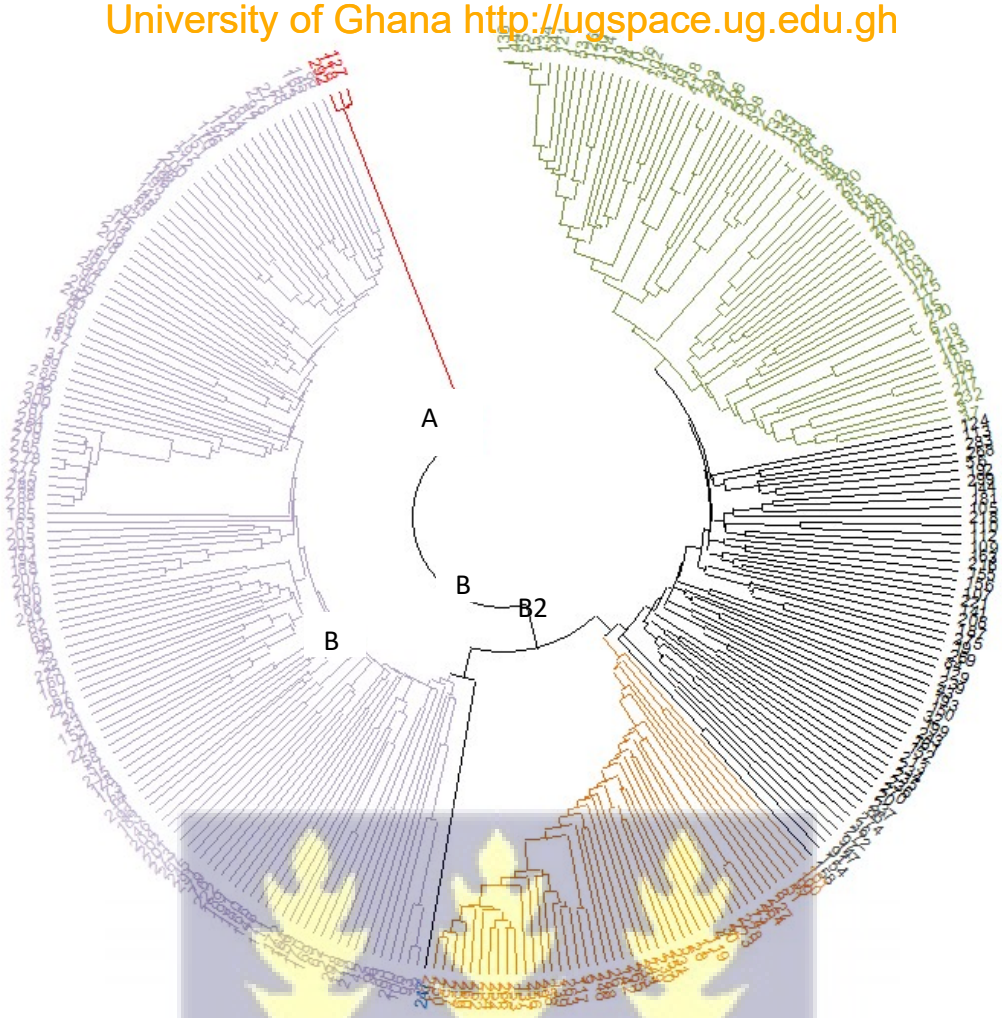


Fig 4.4 Hierarchical clustering of Mungbean genotypes based on 5,037 SNPs.

### 4.3.3 Population structure and Principal Component Analysis

4.3.3.1 Principal Component Analysis

Principal component analysis results based on the 5,037 SNPs further explained the grouping seen in the population structure (Fig.4.5). The first and second PCs explained 14.3% and 8.6 % of the SNP variation respectively. The PCA biplot explains 14.3% and 8.6% of the total variation by the first and second principal components. It shows the distribution of genotypes based on genetic variation. Clustering in the PCA plot indicates genetic similarities, aiding in the selection of diverse parental lines for breeding. PC1 (14.3% EVP) captures the most significant variation in the mungbean genotypes, potentially related to specific traits or

genetic markers while PC2 (8.6% EVP) captures the second most significant source of variation, which might relate to another set of traits or markers



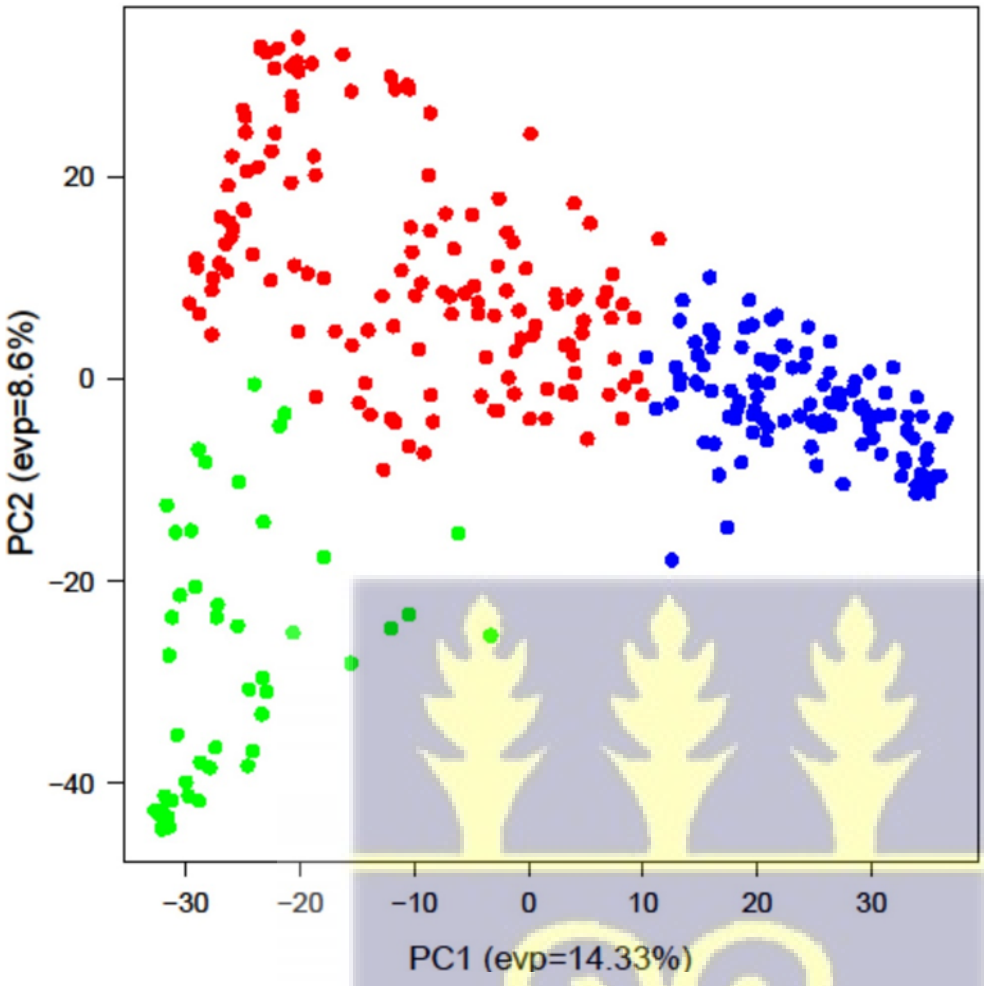


Fig 4.5 principal component analysis biplot

*EVP: explained variance percentage

**4.3.3.2 Population structure**

Based on the ΔK, the output from population structure analysis of the Mungbean revealed the presence of 2 major clusters (k=2) and additional sub-clusters (k=8) (Fig.4.6). Each colour represents a genetic cluster or group. The distinct colours across the bars (K=2, K=4, K=8) indicates the presence of multiple genetic clusters within the populations. Individuals that have mixed colours are admixed, meaning they have genetic contributions from more than one cluster. The colour separations between the clusters (with individuals mainly

belonging to one colour), suggests the presence of strong population differentiation. On the other hand, the significant admixture indicates ongoing gene flow between populations.



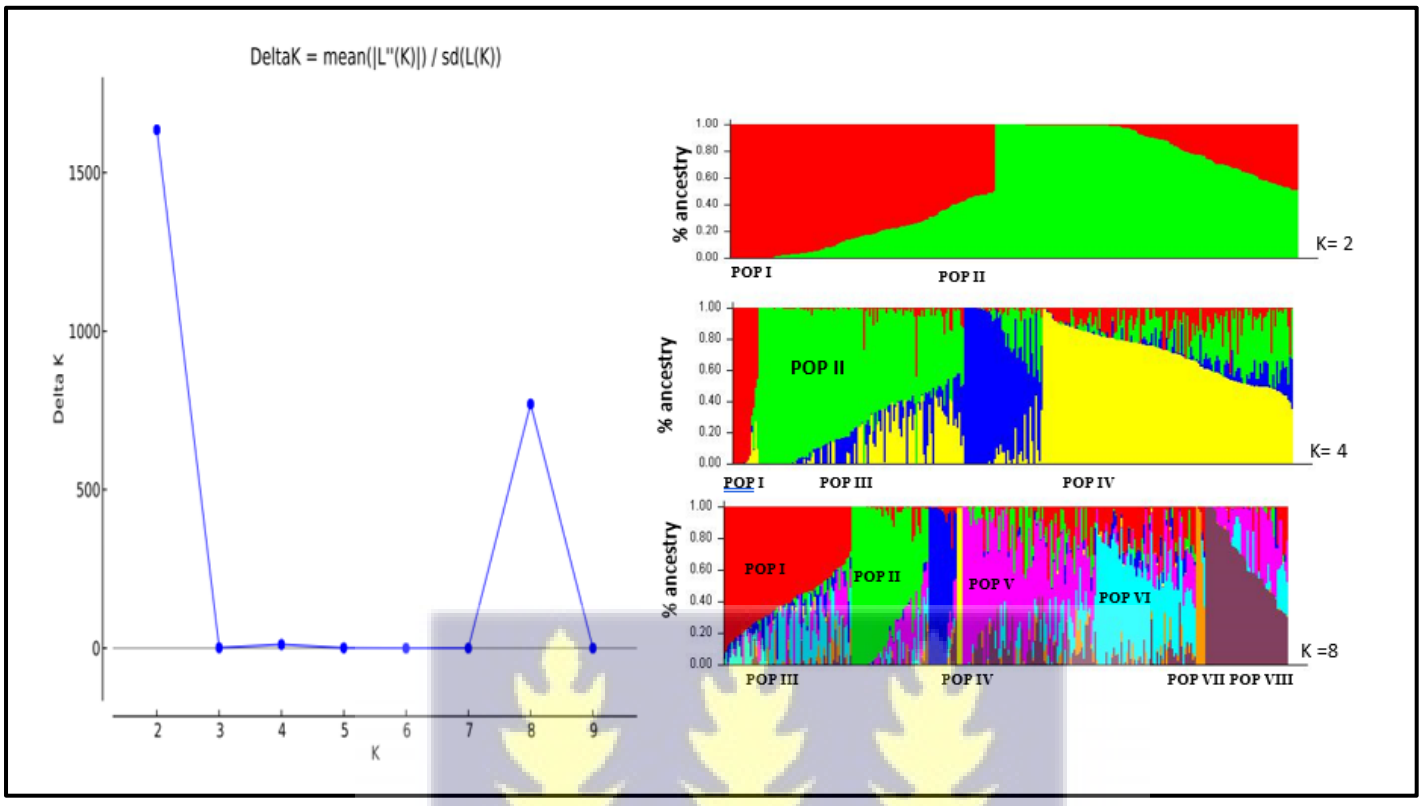


Fig 4. 6. Population structure in the germplasm of mungbean with ΔK displaying the optimal K value (K=2), then (K=8) 5037 SNPs were used to determine the population structure for mungbean genotypes. Every subgroup is symbolized with a distinct colour.

### 4.3.4 Analysis of Molecular Variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was carried out to assess population differentiation in the Mungbean populations using the SNP markers. The results of AMOVA revealed that differences between population accounted for 10% of the variation while the differences within population accounted for 90% of variation accordingly.

Table 4.2 Analysis of molecular variance for genetic differentiation among and within clusters of Mungbean genotypes.

| Source | Df | SS | MS | Est. Var. | % | Stat | Value | P (rand >= data) |
|---|---|---|---|---|---|---|---|---|
| Among Pops | 13 | 26105.976 | 2008.152 | 89.463 | 10% | **PhiPT** | 0.103 | 0.000 |

| | | | | | |
|---|---|---|---|---|---|
| Within Pops | 286 | 223391.250 | 781.088 | 781.088 | 90% |
| Total | 299 | 249497.227 | | 870.551 | 100% |



## 4.4 **Discussion**

Plant breeding relies heavily on genetic diversity because it creates a pool of variation that can be utilized to develop superior cultivars with desired features (Gizebelus et al.,2014). Understanding the genetic array of a mini-core collection is essential for creating high-yielding and disease resistant varieties of mungbean in Africa and Asia (Nair et al., 2022). To form long-term and successful breeding programs, knowledge of the genetic diversity of germplasm is essential. In order to manage, develop genetically and conserve mungbean genotype in Nigeria, we looked at the genetic diversity across some mungbean genotypes from AVRDC mini-core collection introduced into Nigeria for this study. In this work the distribution and degree of genetic diversity among mungbean genotypes gathered from various geographical sources were found to be moderately polymorphic. However, 76% and 63% polymorphism has been reported for mungbean collections from Brazil and Nigeria, according to inter simple sequence repeat (ISSR) markers (Chen et al., 2015). In order to comprehend the genetic linkages among genotypes coming from various geographic sources, the current work employed high-throughput genotyping employing DArTseq SNP markers. A number of significant food crops, including oilseed crops (0.21) (Cruz et al., 2013), soybeans (0.28) (Bisen et al., 2014), cowpeas (0.24) (Sodeji et al., 2021), common beans (0.25) (Nemli et al., 2017), and chickpeas (0.32) (Farahani et al., 2019), also exhibited moderate polymorphism. When Fatokun et al. (2018) examined 370 cowpea genotypes, they too found similar results. Similarly, the USDA cowpea germplasm showed low genetic distance and minimal variability (Wang et al., 2016). More accessions from different sources are indicative of more genetic variety, according to Muñoz-Amatriaín et al. (2017). Furthermore, the low genetic diversity of mungbean may be caused by its reproductive biology rather than merely having a narrow background because it is a self-pollinating crop. Nigeria's efforts to breed mungbean may be impacted by the study's conclusions of genotype-specific genetic variability in the crop. Low genetic variety may restrict the potential for development, but high genetic diversity between genotypes offers a vast pool of variation for creating superior

cultivars (Asiedu et al., 1998). These findings can be useful when making important breeding choices and choosing parent lines for crossbreeds to achieve certain breeding objectives.

The summary of SNP data per chromosome (Table 4.1), reveals the values of key metrics like gene diversity (GD), polymorphic information content (PIC), minor allele frequency (MAF), and observed (Ho) and expected heterozygosity (He). Gene Diversity (GD) values (0.33), indicates moderate genetic variation within the population. PIC values (0.27), which reflect the discriminatory power of markers suggests that the SNPs are moderately informative for genetic studies. Minor Allele Frequency (MAF), ranging from 0.23 to 0.27, indicates the distribution of less common alleles in the population while relatively even MAF suggests a well-balanced allele distribution across genotypes. The difference between observed heterozygosity (Ho) and expected heterozygosity (He) suggests potential inbreeding or population structure, as Ho is consistently lower than He, which can be due to non-random mating or selective breeding efforts (Fatmawati *et al*., 2021). The relatively low levels of heterozygosity (Figure 4.2) suggest that the population is largely homozygous, which may be a result of selective breeding or limited genetic introgression from other populations. This is a crucial observation, as higher heterozygosity is often associated with greater genetic resilience and adaptability(Teeken *et al*., 2018).

The heatmap of the kinship matrix using average linkage clustering based on SNP markers (Figure 4.3) visualizes the genetic relatedness between genotypes, identifying clusters that reflect shared ancestry or breeding histories. The distinct clusters indicate varying degrees of genetic similarity, where closely related individuals are grouped together. This visualization helps identify genetically diverse lines that could be useful for breeding programs aimed at enhancing genetic diversity and introducing new traits(Hartigan, 1985).

The hierarchical relationships between the mungbean genotypes (Figure 4.4), revealed clusters based on their genetic similarity while also revealing potential shared ancestry between some of the genotypes. This type of clustering is invaluable in identifying genetically distinct groups for breeding, (Patterson et al., 2006). Genetically diverse clusters, as shown in the dendrogram, are essential for creating genetic variability in breeding programs. Crossing genetically diverse parents often results in offspring with desirable traits, such as improved yield, disease resistance, or climate resilience (Patterson et al., 2006). Genetic diversity within

mungbean has been a focus of numerous studies, highlighting the importance of diverse SNP profiles in breeding programs (Nair et al., 2019). Well-separated clusters in the dendrogram may signify distinct genotypic groups, potentially shaped by different selection pressures, breeding histories, or geographic isolation (Hartigan, 1985). Such patterns are important for identifying genotypes adapted to different environmental conditions or stressors, which can be strategically used in future breeding programs (Rohlf & Sokal, 1981).

The PCA plot shows the distribution of genotypes based on their genetic similarities (Figure 4.5). Genotypes that cluster closely together are genetically more similar, suggesting they share common ancestry or traits (Reddy et al., 2020). This clustering can be used to identify potential parental lines for breeding programs aimed at enhancing desirable traits like disease resistance, yield, or environmental adaptability(Mishra et al., 2024). The distribution of genotypes across the PCA axes highlights the presence of genetic diversity, which is crucial for breeding programs. PC1, which explains the largest proportion of genetic variation (14.3%), likely reflects genetic markers associated with key traits such as seed size, growth rate, or pest resistance. PC2 (8.6%) captures another dimension of genetic diversity, potentially reflecting other important agronomic traits, Similar findings were reported by Vu et al., (2022) and Kang et al. (2014) who employed PCA to illustrate the genetic relationships among mungbean lines, revealing distinct clusters that corresponded to geographic origins and specific traits and also having the first two principal components accounting for a significant portion of the genetic variation.

Understanding the population structure is essential for plant breeding programs. In mungbean, identifying distinct genetic clusters can help breeders select parent plants from different populations to maximize genetic diversity and improve traits like disease resistance or yield stability(Giang et al., 2024). The population structure analysis (figure 4.6) provides insights into the genetic relationships among the sampled populations. The presence of well-defined clusters suggests genetic differentiation due to factors like geographic isolation, selection pressures, or domestication. These findings align with previous studies of Singh & Sharma, (2014) that used molecular markers to assess genetic diversity in mungbean and other legume crops, their STRUCTURE analysis revealed clear differentiation between landraces from different geographic regions, and this is crucial for understanding domestication patterns and improving crop varieties. By analyzing genetic

structure, breeders can strategically introduce genetic variability into breeding lines, promoting resilience and adaptability. The integration of this knowledge into breeding programs could lead to the development of superior mungbean varieties with better adaptation to diverse environments and increased yield stability(Giang et al., 2024)

The AMOVA results showing 10% variation among populations and 90% within populations highlights the significance of intra-population diversity and important implications for understanding the species' genetic structure. The moderate genetic variation among populations, as indicated by the PhiPT value of 0.103, suggests that populations are not completely isolated but share a significant amount of genetic material. This could be due to factors like gene flow or shared evolutionary history among populations, which maintains genetic similarities across different regions (Kardos et al., 2021). The finding that 90% of the genetic variation occurred within mungbean populations is consistent with similar studies by (Mwangi et al., 2021) on Kenyan mungbean genotypes which indicated that 99% of genetic variation was within populations, implying strong genetic diversity within individual populations. This trend has also been observed in studies across other legumes and crops, where the majority of genetic variation is concentrated within populations rather than between them (Gwag et al., 2010).This has practical implications for breeding programs, as it suggests that efforts focused on within-population selection may be more productive for improving traits like yield and disease resistance (Pandey et al., 2024). Additionally, this pattern of genetic variation suggests that conservation strategies for mungbean should prioritize maintaining genetic diversity within populations, as that is where most of the variation resides(Avramidou et al., 2024). Populations that appear genetically similar on a macro scale may still contain valuable diversity at the individual level, which is crucial for adaptation to environmental changes and long-term species survival (Beckman et al., 2020).

### 4.5 Conclusion

This study provides a comprehensive assessment of the genetic diversity and population structure of mungbean (Vigna radiata) genotypes using DArTseq-derived SNP markers, offering critical insights for breeding and conservation efforts. The analysis of 5,037 high-quality SNPs across 293 genotypes revealed moderate genetic diversity, with distinct clustering patterns that highlight the existence of genetically diverse

and closely related accessions. The AMOVA results, showing 90% variation within populations and 10% among populations, emphasize the predominance of intra-population diversity, making within-population selection an effective strategy for genetic improvement. The principal component and clustering analyses further delineated two major genetic clusters, with sub-clusters indicating varying degrees of genetic differentiation. These findings suggest that while some genotypes exhibit strong genetic relatedness, others possess unique allelic variations that can be strategically utilized in breeding programs. The observed low heterozygosity suggests that mungbean populations may have undergone selection bottlenecks or inbreeding, necessitating the introduction of new genetic materials to enhance diversity and resilience. This study underscores the potential of SNP-based characterization in identifying valuable genetic resources for mungbean improvement. The identification of polymorphic markers, genetic clusters, and diverse accessions provides a roadmap for selecting superior parental lines with desirable agronomic traits, such as disease resistance, stress tolerance, and yield enhancement. Moving forward, integrating genomic selection, gene mapping, and targeted hybridization strategies will be crucial in accelerating mungbean genetic gains and varietal development. These findings serve as a foundation for future breeding efforts, ensuring the sustainable cultivation of high-yielding, resilient mungbean varieties in diverse agroecological conditions.

# CHAPTER FIVE

## 5.0 Multi-Location Evaluation of Mungbean Genotypes in Nigeria

### 5.1 Introduction

Interest in mung bean cultivation has been increasing in Nigeria because of its potential to enhance food security, improve soil fertility, and provide nutritional benefits, particularly in regions with marginal agricultural conditions. Evaluation of mungbean genotypes across multiple locations is essential to identify genotypes that exhibit stability and high performance under varying environmental conditions. Multi-location trials help to understand genotype-by-environment interactions, which are critical for selecting genotypes with broad adaptability and specific suitability to different ecological zones (Lin *et al*., 1986). Such evaluations are particularly relevant in Nigeria, where diverse agroecological zones present unique challenges and opportunities for crop production. Several studies have underscored the importance of multilocation trials in mung bean breeding programs. For instance, Asante et al. (2008) emphasized that evaluating genotypes in different environments helps identify traits that contribute to yield stability and resilience against biotic and abiotic stresses. This approach ensures that the selected genotypes perform well across a range of conditions, thereby increasing the likelihood of successful cultivation and adoption by farmers.

The establishment of multi-location trials is not only beneficial for yield assessment, but also for understanding the adaptability of different genotypes to varying environmental stresses. Yan (2019) has shown that delineating mega-environments based on GE patterns can lead to more effective breeding strategies and improved crop variety. This approach is particularly relevant in Nigeria, where climatic variability poses challenges for consistent crop production.

The main objective of this study was to assess the impact of genotype-by-environment interactions on growth, seed yield and protein of mungbean in four diverse test environments in Nigeria.

### 5.2 Materials and methods

### 5.2.1 Locations of study



The study was conducted at two locations representing contrasting agro-ecological and climatic conditions in southern Nigeria: Awka (6°14′N, 7°04′E; altitude 103 m) in Anambra State, and Uyo (5°03′N, 7°56′E; altitude 65 m) in Akwa Ibom State. Both sites are characterized by bimodal rainfall patterns, but differ in mean annual precipitation and temperature regimes. Awka has a derived savanna climate with moderate rainfall (about 1800 mm annually) and relatively high temperatures during the dry season, while Uyo lies in the humid rainforest zone with higher rainfall (about 2300 mm annually) and more stable humidity levels. Trials were carried out across four environments representing combinations of location and season: Awka Rainy Season (AwkaRain), Awka Dry Season (AwkaDry), Uyo Rainy Season (UyoRain), and Uyo Dry Season (UyoDry). These environments were selected to capture naturally occurring abiotic stress contrasts such as moisture deficit, temperature variation, and photoperiod differences typical of Nigeria's tropical production systems.

Table 5.1 Geographical characteristics of study environments

| Environment | Location | Latitude | Longitude | Altitude(m) | Cropping Season |
|---|---|---|---|---|---|
| Awka Dry (E1) | Anambra State | 6.2530°N | 7.1150° E | 90.75 m | 2022 |
| Awka Rain (E2) | Anambra State | 6.2530°N | 7.1150° E | 90.75 m | 2023 |
| Uyo Dry (E3) | Akwaibom State | 5.0382°N | 7.9767° E | 18.45 m | 2022 |
| Uyo Rain (E4) | Akwaibom State | 5.0382°N | 7.9767° E | 18.45 m | 2023 |

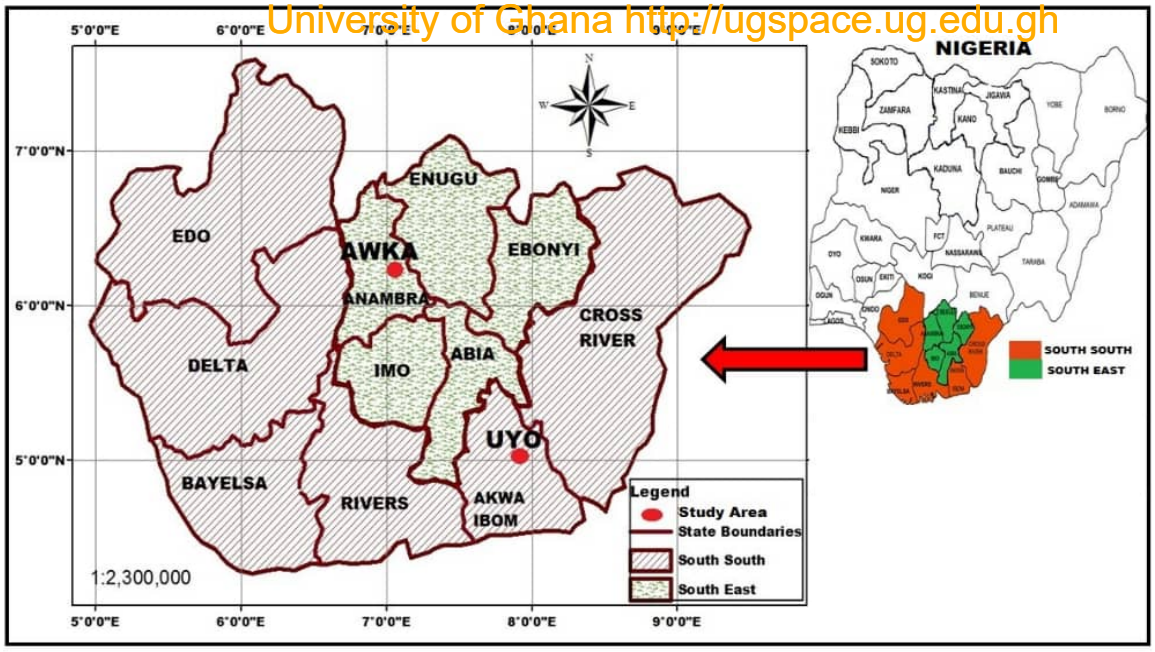


Fig 5.1 Map of southern Nigeria showing study locations

### 5.2.2 **Experimental materials and field design**

A total of 120 mungbean (*Vigna radiata* (L.) R. Wilczek var**.** *radiata*) genotypes were evaluated. The materials were introduced from the World Vegetable Center (WorldVeg) mini-core collection (Schafleitner et al. 2015) and represent a broad range of genetic diversity for agronomic and seed quality traits. Seeds were multiplied locally before experimentation to ensure uniform seed quality. Each trial was laid out using a 10 × 12 alpha lattice design with two replications per environment. Each experimental unit consisted of a single 4-m row spaced at 50 cm between rows and 10 cm within rows, giving a population density of approximately 200,000 plants $ha^{-1}$. Standard cultural practices were applied uniformly across locations. Manual weeding was carried out as needed, and no irrigation was provided during dry-season trials to expose genotypes to natural moisture stress conditions. Soil samples were collected from each environment prior to planting to determine basic physicochemical properties. No major pest or disease outbreaks were recorded during the experiment, though prophylactic insecticide application was carried out at 14 and 28 days after emergence.

### 5.2.3 Data collection 

For each trait, five randomly selected plants from each experimental unit (plot) were used.

The traits measured were as follows:

i. Days to 50% flowering (D2flw): the number of days from sowing until 50% of the plants have at least one open flower was estimated and recorded

ii. Plant height (PH) at 6Weeks after sowing: The plant height was measured using meter rule at 6 weeks

iii. Estimation of grain yield and 100-seed weight: 100 seeds were manually counted and weighed in triplicate. Grain yield was recorded in grams per plot in individual replicates and converted to kg ha−1. Border rows were excluded from the plot yield estimation to eliminate border effects.

**iv.** Protein Content**:** Mungbean samples were analyzed for protein content using the Kjeldahl method (AOAC, 2005). Grain samples were digested using concentrated sulfuric acid and a catalyst mixture. The nitrogen content was determined by distillation and titration, and protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25 (Mosse, 1990)

### 5.2.4 Statistical Analysis

Data from all environments were subjected to combined analysis of variance (ANOVA) using the linear mixed model (LMM) implemented in the R package (R Core Team, 2018), of the form;

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + R_{(k)j} + \beta_{b(kj)} + \varepsilon_{ijk} \quad - \quad - \quad - \quad (1)$$

where , $Y_{ijkl}$= observed genotype i in the environment j, replicate k and block b, $\mu$= overall mean; $G_i$=fixed effect of genotype i; $E_j$= random effect of environment j; $GE_{ij}$ = random genotype by environment interaction; $R_{(k)j}$=random replication effect nested within environment j; $\beta_{b(kj)}$=random block effect b nested within replication k and environment j( alpha lattice blocking); $\varepsilon_{ijk}$= residual error

The resulting mean data from the analysis of variance were subsequently employed in AMMI analysis (Gauch, 2006) to assess the stability of the genotypes. This analysis was conducted using the "Metan" package in the R software (Olivoto and Lúcio, 2020), utilizing the following model:

$$Y_{ijk} = \mu + G_i + E_j + \sum_{k=1}^{M} \lambda_k \times \alpha_{ik} + \gamma_{jk} + \rho_{ij} \quad (2)$$



where $Y_{ijk}$ represents the yield of the ith genotype in the jth environment. Gi denotes the effect of the ith genotype (calculated as the genotype mean minus the grand mean), whereas Ej signifies the effect of the jth environment (environment mean minus the grand mean). $\lambda_k$ is the square root of the eigenvalue for the kth Interaction Principal Component (IPCA) axis. $\alpha_{ik}$ and $\gamma_{jk}$ are the principal component scores for IPCA axis k of the ith genotype and jth environment, respectively. where ρij indicates the deviation of the ith genotype in the jth environment from the model.

To identify mega-environments and illustrate the "which won where" pattern, a genotype plus genotype-vs-environment interaction (GGE) analysis was conducted using the "Metan" package in R software (Olivoto and Lúcio, 2020). The GGE biplot was constructed using singular value decomposition (SVD) of the principal components, as outlined by (Yan and Tinker (2006). The following GGE model was employed:

$$y_{ij} = \mu + \beta_j + \Sigma k\, \lambda k\, \alpha ik + \varepsilon_{ij} \quad (3)$$

In this equation, yij represents the performance of the i-th genotype in the j-th environment, μ is the overall mean, βj denotes the main effect of the j-th environment, k indicates the number of principal components (PC), λk is the singular value of the k-th PC, αik and  are the PC scores for the i-th genotypes and j-th environment, respectively, and $\varepsilon_{ij}$ represents the residual associated with the i-th genotype and j-th environment.

To identify mega-environments within the experimental area, "which won where" scatter plots were created. This visualization included a polygon drawn using symmetrical scaling, linking distant genotypes from the biplot and enclosing all genotypes. The polygon was subsequently divided by perpendicular lines, extending from the origin of the biplot to its edges (Yan and Tinker, 2006). The environmental vectors are projected from the axis. A ranking plot based on mean versus stability was generated using symmetrical scaling, employing the average environment coordinate (AEC) concept to draw the average line and an arrow line indicating increasing yield mean performance (Yan and Rajcan, 2002). Additionally, a comparison plot of

genotype ranking relative to an ideal genotype was produced using symmetrical scaling by applying the same AEC concept to compare genotypes to a hypothetical ideal genotype.

## 5.3 Results

### 5.3.1 Agronomic performance of mungbean genotypes

The combined analysis across the four environments revealed highly significant ($p < 0.0001$) effects of genotype (G), environment (E), and genotype-by-environment interaction (GxE) for yield, plant height, days to 50% flowering, and protein content (Table 5.2).

Table 5.2: Mean squares for yield, plant height, days to flowering and seed protein content

| Source of Variation | df | Yield | PHT | DT50%F | Protein |
|---|---|---|---|---|---|
| Genotype (GEN) | 119 | $1.125 \times 10^{8}$** | 5288.66* | 1279.46* | 70.16* |
| Environment (ENV) | 3 | $6.749 \times 10^{8}$** | 51239.33* | 147.87* | 0.22** |
| GEN × ENV | 357 | $2.445 \times 10^{8}$** | 13263.75* | 3853.13* | 19.02* |
| Residual | 480 | $2.202 \times 10^{8}$ | 16407.57 | 3912.00 | 26.67 |

df-degree of freedom, PHT- Plant height, DT50%F-days to fifty percent flowering

### 5.3.2 Relative contributions of the four measured traits to variability across environments

The first principal component (PC1) explained 37.43% of the total variability and was dominated by yield and plant height, suggesting that PC1 primarily represents plant growth and productivity (Table 5.3). PC2, explaining 25.31% of the variance, was strongly influenced by protein content and days to 50% flowering, indicating that PC2 may represent maturity and protein accumulation. PC3, explaining 24.65% of the variance, showed an opposing effect of days to 50% flowering and protein content, possibly distinguishing early versus late-maturing genotypes. PC4, explaining 12.62% of the variance, exhibited a high influence of yield (0.707) and plant height (-0.704), likely representing a contrast between yield and height.

Table 5.3: Trait contributions to total variability



| Trait | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Days to 50% flowering | -0.081 | 0.681 | 0.725 | 0.055 |
| Yield | 0.706 | -0.020 | 0.044 | 0.707 |
| Plant height | 0.703 | 0.064 | 0.072 | -0.704 |
| Protein | 0.033 | 0.729 | -0.683 | 0.030 |
| Cummulative Percentage | 37.4 | 62.7 | 87.4 | 100.0 |

### 5.3.3 Additive Main Effect and Multiplicative Interaction effects for the studied traits

The AMMI analysis further confirmed the significant G, E, and GxE effects (Table 5.4). The AMMI biplots effectively visualized GxE interactions (Figures 2 & 3 in the attached document), revealing varying genotype stability across environments. According to Table 5.3 in the attached document, Genotypes 130, 105 and 20 demonstrated superior and stable performance across environments. UyodryS1 supported the highest yields (1111 kg/ha), protein content (23.02%), and plant height (24.05 cm) compared to other tested environments.

### 5.3.4 Environments' mean traits and stability index (IPCA 1) scores evaluated across four test environments in Nigeria

The environment means for the four traits and their stability index (IPCA1) are represented in Table 5.5. Uyo Rain has the highest yield (2341.79) and the highest stability (IPCA1: 1230.88). Uyo Dry is least stable for days to 50% flowering (0.664), indicating flowering time is more variable there. Plant Height_IPCA1 shows that Uyo Rain has the most variable plant height (11.99), while Awka Rain is more stable (-5.92). Protein stability is relatively low across environments, indicating small differences in variation.

Table 5.4. AMMI analysis of 120 mungbean genotypes evaluated in four test environments within Nigeria

| Trait | Source | Df | Sum of Squares | Mean Square | F-Value | P-Value |
|---|---|---|---|---|---|---|
| Yield | Genotype (G) | 119 | $1.125 \times 10^{8}$ | 945378.151 | 2.061 | $4.05 \times 10^{-8}$ |
| | Environment (E) | 3 | $6.749 \times 10^{8}$ | $2.250 \times 10^{8}$ | 490.429 | $1.01 \times 10^{-145}$ |
| | G × E Interaction | 357 | $2.445 \times 10^{8}$ | 685034.179 | 1.493 | $2.23 \times 10^{-5}$ |
| | IPC1 | 122 | $1.143 \times 10^{8}$ | 936558.452 | 2.042 | $4.78 \times 10^{-8}$ |
| | IPC2 | 121 | $5.631 \times 10^{7}$ | 465334.625 | 1.014 | 0.449 |
| | IPC3 | 120 | $5.893 \times 10^{6}$ | 49107.848 | 0.107 | 1.000 |
| | Residual | 480 | $2.202 \times 10^{8}$ | 458751.492 | - | - |
| Plant Height | Genotype (G) | 119 | 5288.66 | 44.45 | 1.300 | $2.96 \times 10^{-2}$ |
| | Environment (E) | 3 | 51239.33 | 17079.78 | 499.665 | $3.42 \times 10^{-147}$ |
| | G × E Interaction | 357 | 13263.75 | 37.14 | 1.087 | $1.98 \times 10^{-1}$ |
| | IPC1 | 122 | 4246.68 | 34.81 | 1.018 | 0.438 |
| | IPC2 | 121 | 2447.22 | 20.22 | 0.592 | 0.999 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | IPC3 | 120 | 1590.15 | 13.25 | 0.388 | 0.999 |
| | Residual | 480 | 16407.57 | 34.18 | - | - |
| Protein Content | Genotype (G) | 119 | 70.16 | 0.59 | 10.609 | $2.39 \times 10^{-80}$ |
| | Environment (E) | 3 | 0.22 | 0.07 | 1.310 | $2.70 \times 10^{-1}$ |
| | G × E Interaction | 357 | 19.02 | 0.05 | 0.959 | $6.62 \times 10^{-1}$ |
| | IPC1 | 122 | 36.82 | 0.30 | 5.432 | $1.11 \times 10^{-16}$ |
| | IPC2 | 121 | 4.19 | 0.03 | 0.623 | 0.999 |
| | IPC3 | 120 | 1.96 | 0.02 | 0.294 | 1.000 |
| | Residual | 480 | 26.67 | 0.06 | - | - |
| Days to 50% Flowering | Genotype (G) | 119 | 1279.46 | 10.75 | 1.319 | $2.31 \times 10^{-2}$ |
| | Environment (E) | 3 | 147.87 | 49.29 | 6.048 | $4.77 \times 10^{-4}$ |
| | GEN × ENV | 357 | 3853.13 | 10.79 | 1.324 | $2.12 \times 10^{-3}$ |
| | IPC1 | 122 | 2370.98 | 19.43 | 2.385 | $2.53 \times 10^{-11}$ |
| | IPC2 | 121 | 109.06 | 0.90 | 0.111 | 1.000 |
| | IPC3 | 120 | 86.13 | 0.72 | 0.088 | 1.000 |
| | Residual | 480 | 3912.00 | 8.15 | - | - |

Table 5.5 Environments' mean traits and stability index (IPAC 1) scores evaluated across four test environments in Nigeria

| Environment | DT50F | DT50F_IPCA1 | YIELD | YIELD_IPCA1 | PHT | PHT_IPCA1 | Protein | Protein_IPCA1 |
|---|---|---|---|---|---|---|---|---|
| AwkadryS1 | 40.16 | -0.156 | 249.43 | -861.48 | 18.19 | -5.87 | 23.02 | 0.0033 |
| AwkarainS1 | 39.96 | -0.356 | 1419.6 | 308.68 | 18.13 | -5.92 | 23.02 | -0.0037 |
| UyodryS1 | 40.98 | 0.664 | 432.83 | -678.08 | 23.84 | -0.21 | 23 | -0.0208 |
| UyorainS1 | 40.17 | -0.152 | 2341.79 | 1230.88 | 36.05 | 11.99 | 23.04 | 0.0213 |

### 5.3.5 Description of AMMI and GGE biplots Plots for the studied traits

The AMMI1 biplots (Figure 5.2) display the GxE interaction for yield, plant height, days to 50% flowering, and protein content. Genotypes and environments are plotted based on their mean performance and IPCA1 scores. Genotypes close to the origin show stability across environments. Genotypes 130, 105, and 20 are relatively close to the origin in the yield biplot, indicating their stability across environments. Environments close to specific genotypes show higher performance of those genotypes. UyoRain appears associated with genotypes clustered in its direction, suggesting they performed relatively well in UyoRain for yield. Plant height stability is observed similarly, with different genotypes showing more stable performance.

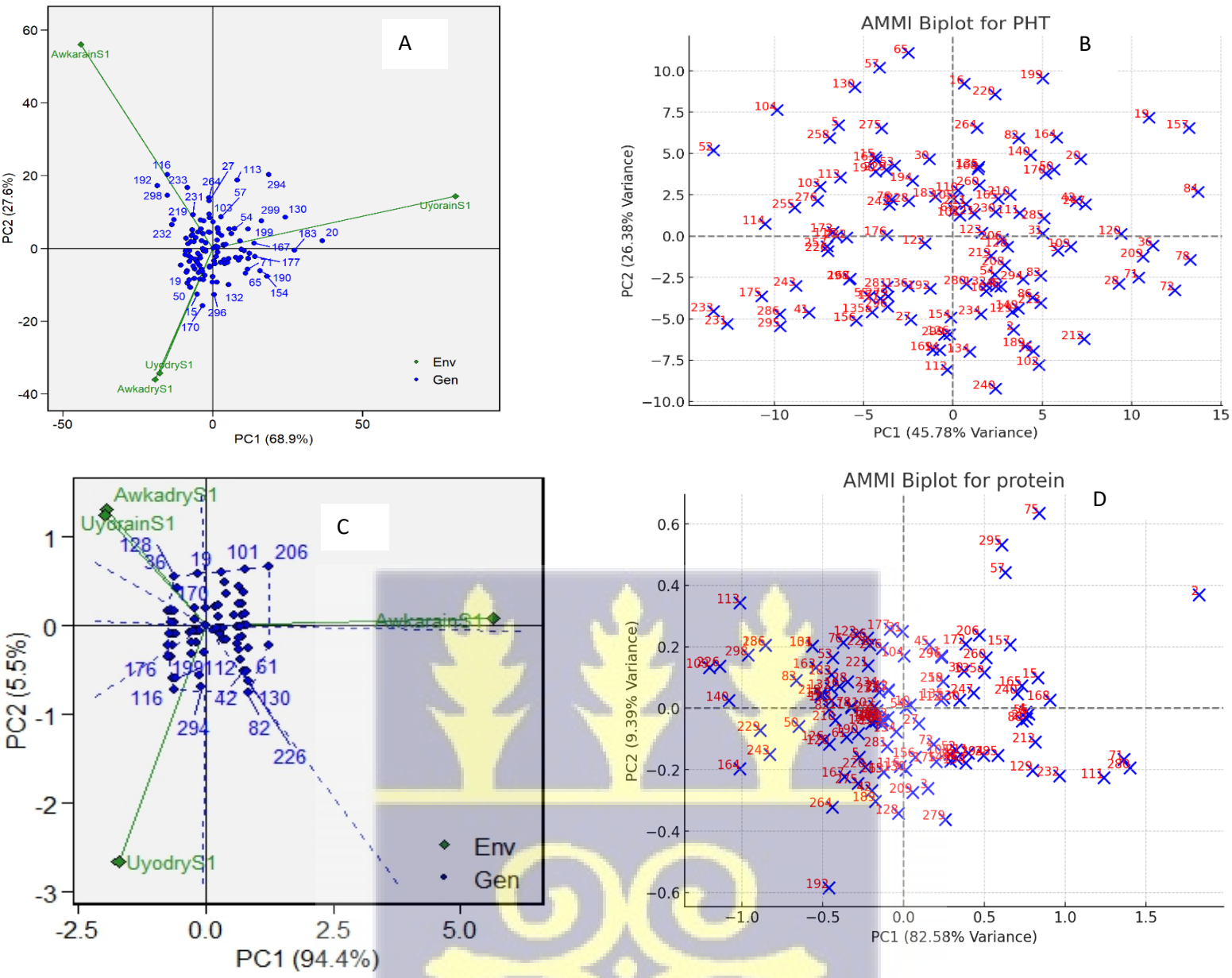


**Figure 5.2: AMMI1 biplot for (A) mean yield, (B) Plant height, (C) Days to fifty percent flowering, (D) Protein content and their respective PC 1 scores for 120 mungbean genotypes evaluated in four environments**

### 5.3.5.1 GGE Biplot for Stability

This GGE biplot visualizes the stability of genotypes across the four environments. Genotypes that cluster tightly together are similarly affected by environmental conditions. Genotypes 130, 105, and 20 are very stable, as they're very close to zero on the biplot. The environments also cluster tightly, indicating that the test locations provide similar environmental conditions

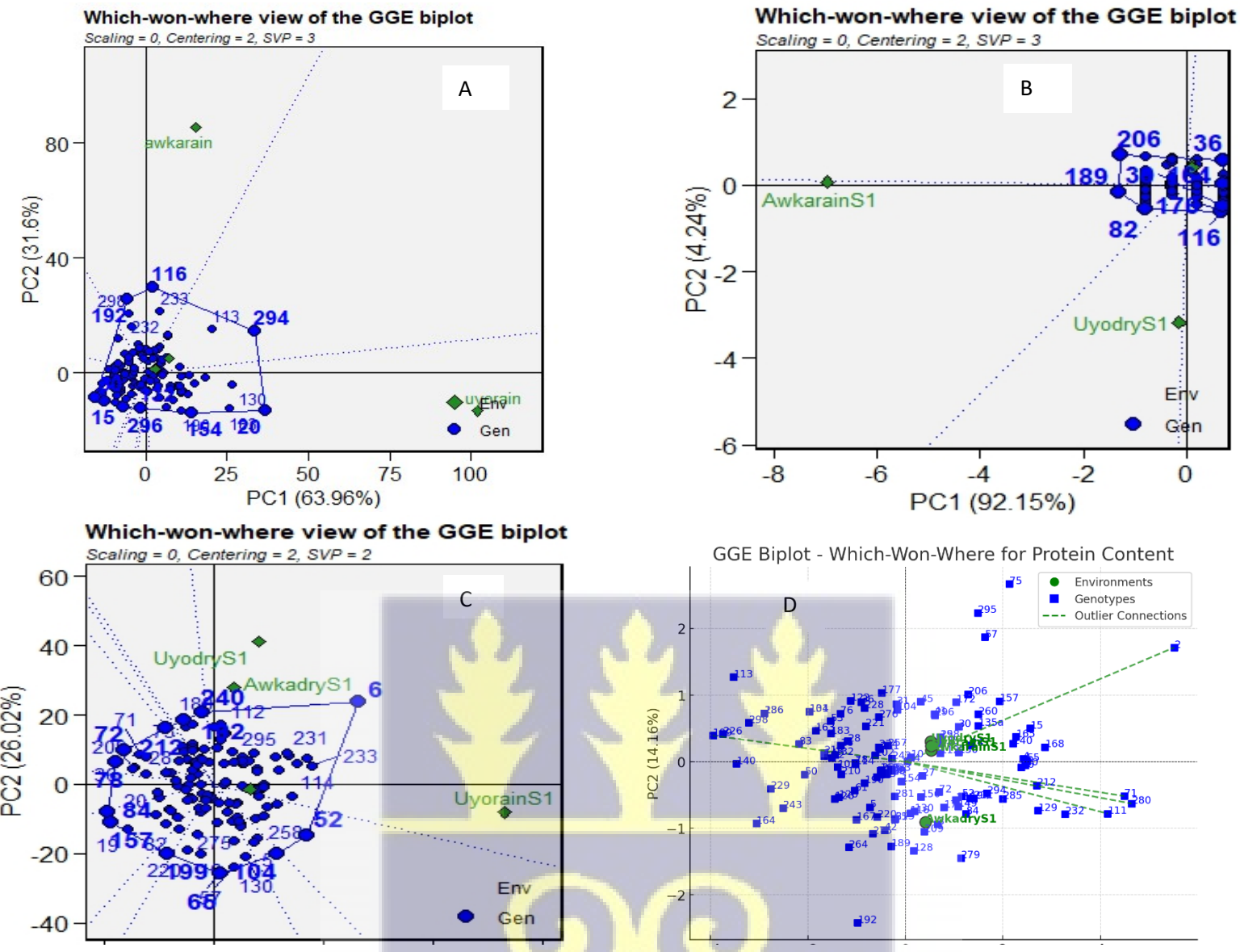


**Figure 5.2: GGE biplot showing which-won-where for Stability for (A) Yield, (B) Days to 50% flowering, (C) Plant Height, (D) Protein content and for 120 mungbean genotypes evaluated in four environments**

### 5.3.5.2 GGE Biplot Showing "Which-Won-Where"

This biplot depicts mega-environments, showcasing the best-performing genotypes in specific environments. The vertex genotypes for the mega-environments represent the best-performing genotypes in those specific environments. This provides insights into which genotypes excel under specific environmental conditions, aiding in targeted breeding strategies. A clear "which-won-where" pattern is evident, showing the specific adaptations of genotypes to particular environments

### 5.3.5.3 GGE Biplot Showing Environment Ranking

The rankings of the test environments based on their ability to discriminate among genotypes for yield, plant height, days to 50% flowering, and protein content is presented in Figure 5.5. Environments closer to the average environment coordinate (AEC) axis better differentiate the genotypes based on yield performance. UyoRain and AwkaDry show longer vectors and are more discriminating, suggesting these environments are more effective in differentiating the yield performance of the mungbean genotypes.

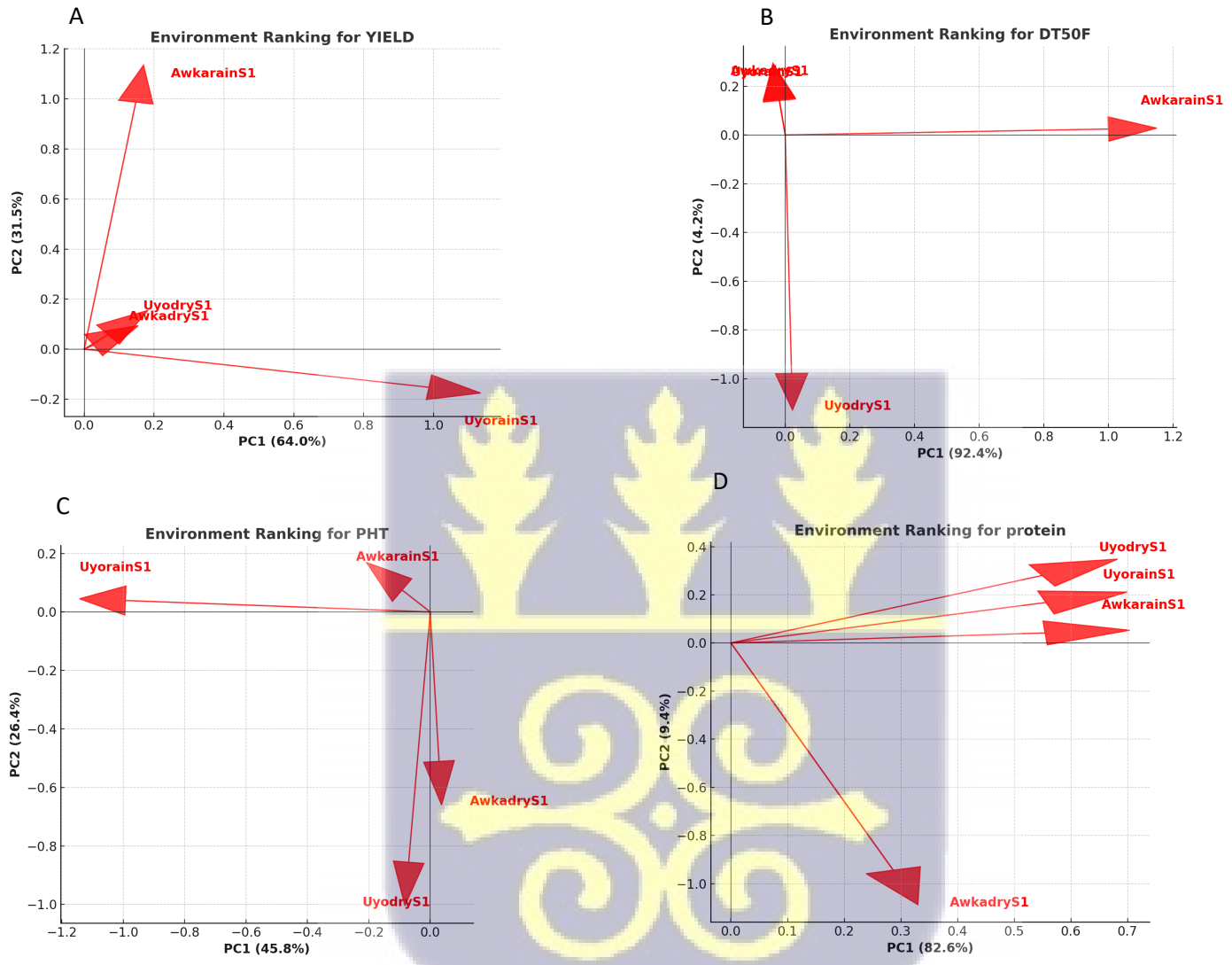


**Figure 5.5** GGE biplot showing Environment ranking for Yield (A), Days to 50% flowering (B), Plant Height(C), Protein content (D) and for 120 mungbean genotypes evaluated in four environments

### 5.3.5.4 GGE Biplot Showing Genotype Ranking



Figure 5.6 shows the ranks of the genotypes according to their mean performance and stability for yield, plant

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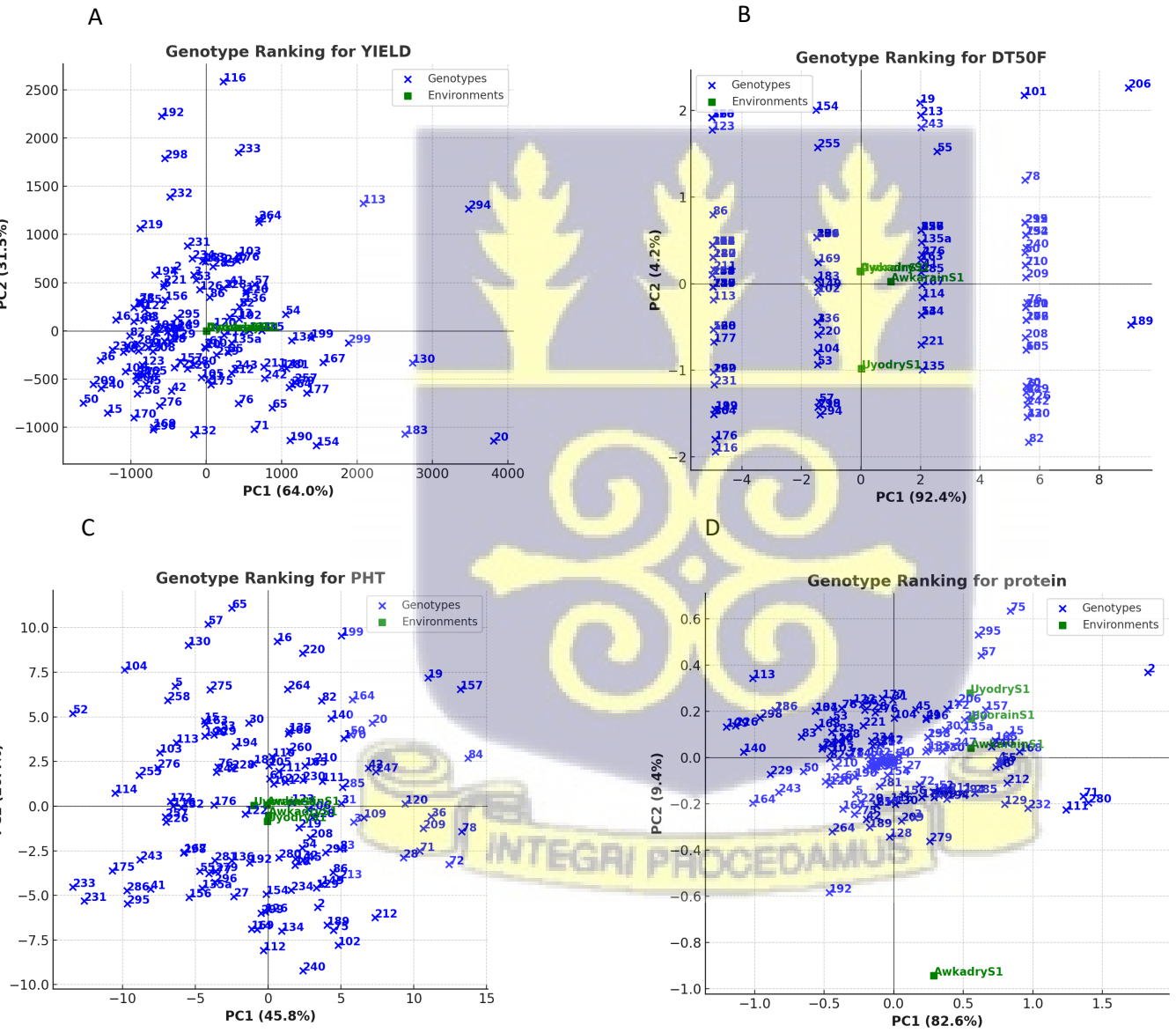


**Figure 5.6** GGE biplot showing Genotype ranking for Yield (A), Days to 50% flowering (B), Plant Height(C), Protein content (D) and for 120 mungbean

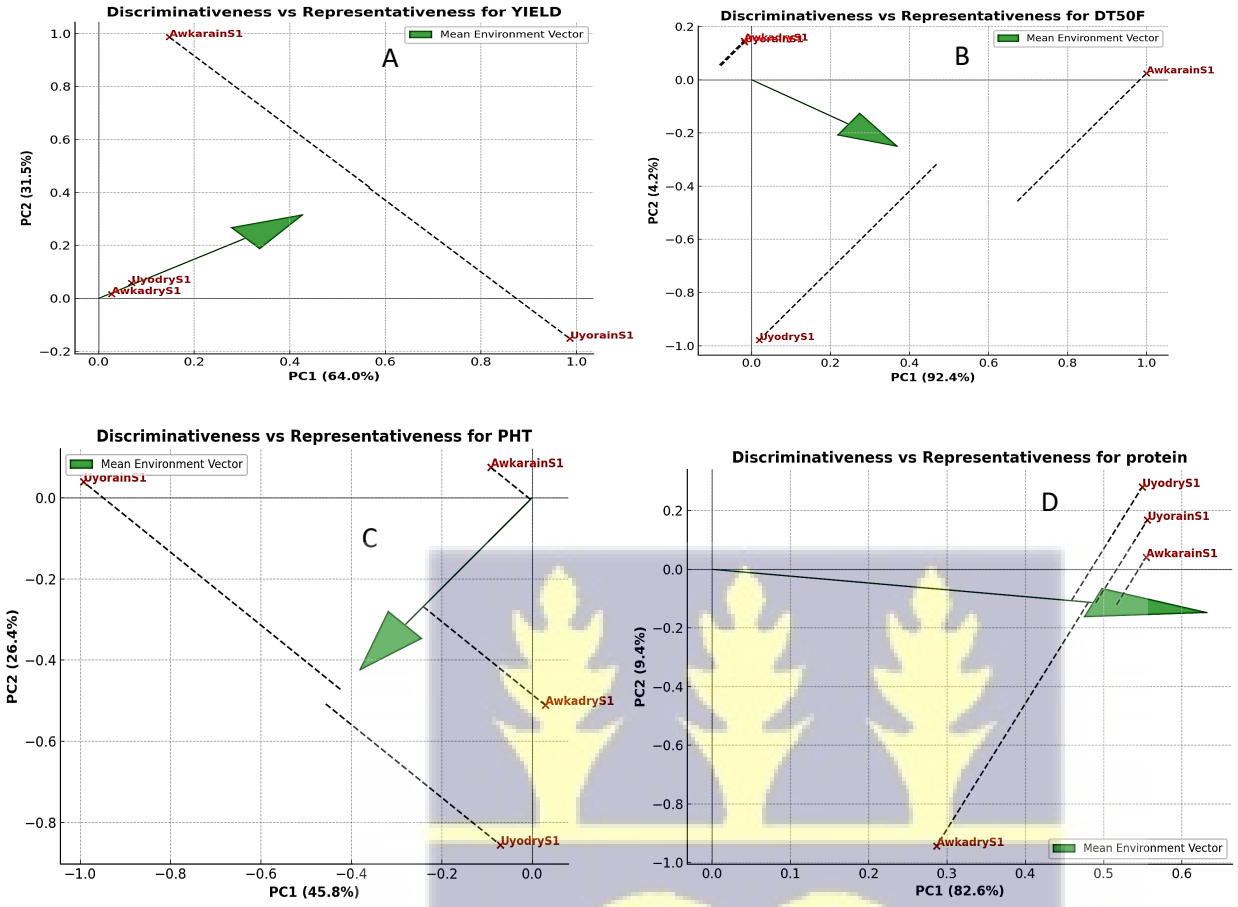


**Figure 8. GGE biplot showing discriminativeness vs representativeness ranking for Yield (A), Days to 50% flowering (B), Plant Height(C), Protein content (D) and for 120 mungbean genotypes evaluated in four environments**

### 5.4 Discussion

Multi-location trials are essential for understanding GxE and identifying stable, high-yielding varieties (Asante et al., 2008; Lin et al., 1986). The significant GxE interactions observed in our study for yield, plant height, days to 50% flowering, and protein content (Table 5.2) underscore the importance of multi-environment testing for mungbean in Nigeria. These findings align with Das et al. (2019), who emphasized the need for multi-environment testing to identify genotypes with durable disease resistance. Specifically, Das et al. (2019) found that responses to Cercospora leaf spot varied significantly across environments, highlighting the importance of evaluating genotypes under diverse conditions. Our results support this, as we found differences in the best-performing mungbean genotypes across locations and seasons in Nigeria, validating the significance of multi-location trials in identifying varieties suitable for diverse agro-ecological zones. The use of AMMI analysis in our study allowed for the effective visualization of GxE interactions (Figures 5.2 and 5.3), facilitating the identification of stable genotypes. Genotypes 130, 105, and 20 consistently demonstrated superior and stable performance across environments. This is consistent with other studies that advocate for the use of AMMI biplots for visualizing GxE interactions (Rao et al., 2022; Tonk et al., 2011; Yan & Tinker, 2006). Rao et al. (2022) used AMMI analysis to identify stable and high-yielding chickpea genotypes across multiple environments in India. Similarly, our study used AMMI analysis to identify stable mungbean genotypes in Nigeria, suggesting that this approach is effective in diverse geographical regions. The superior performance of genotypes 130, 105, and 20 suggests they possess genetic traits conferring adaptability and resilience across varied environmental conditions. Our finding that UyoDry supported the highest yields (1111 kg/ha), protein content (23.02%), and plant height (24.05 cm) underscores the influence of specific environmental conditions on mungbean productivity. This aligns with Uko et al. (2019), who demonstrated that soil amendments significantly improved mungbean yield in southeastern Nigeria. Specifically, Uko et al. (2019) found that applying oil palm bunch ash and poultry manure increased

mungbean yield by up to 40%. While our study did not investigate the effects of soil amendments, the superior performance of mungbean in UyoDry may be attributed to favourable soil conditions, optimal rainfall patterns, or effective management practices. In contrast, lower yields in other environments could result from variations in soil fertility, water availability, pest and disease pressure, or temperature fluctuations. The GGE biplot analysis (Figures 5.4 - 5.7) provided a comprehensive assessment of genotype performance across environments, enabling the identification of mega-environments and superior genotypes for specific locations (Yan, 2019; Yan & Rajcan, 2002). For example, using GGE biplots, as explained by Yan (2019), one can delineate mega-environments based on GE patterns, leading to more effective breeding strategies and improved crop variety selection. Figure 5.4, the "which-won-where" biplot, clearly delineates the best-performing genotypes in each environment, facilitating targeted breeding and agronomic strategies. This finding aligns with Yan's (2019) assertion that delineating mega-environments based on GE patterns leads to more effective breeding strategies and improved crop variety selection. For instance, if Genotype 130 consistently exhibits high performance in UyoDry, it suggests that this genotype is well-suited for cultivation in regions with similar climatic and soil conditions. The PCA results revealed that days to 50% flowering had the largest loading in Component 1 (-0.7169), indicating its significant contribution to variance. This underscores the importance of phenological traits in crop development and adaptation, as highlighted by Mukhthambica *et al*. (2023) emphasized the role of flowering time in determining the adaptation of rice genotypes to different environments. Similarly, our study highlights the importance of days to 50% flowering in explaining the variance in mungbean yield across environments. This suggests that selecting genotypes with appropriate flowering times could be a key strategy for improving yield in different regions of Nigeria.

Moreover, the environment ranking plots (Figure 5.5) provided insights into the discriminating ability and representativeness of the test environments, aiding in the selection of optimal testing locations for future studies. The discriminativeness of UyoRain and AwkaDry, as indicated by their longer vectors, suggests that these environments are more effective in differentiating the yield performance of mungbean genotypes. This information is valuable for breeders in selecting testing locations that provide robust and reliable data for genotype evaluation. Similarly, the representativeness of AwkaRain suggests that this environment is a suitable proxy for broader regional conditions, making it a useful location for preliminary genotype screening.

## 5.6 Conclusion



This study provides a comprehensive evaluation of genotype-by-environment (GxE) interactions in 120 mungbean genotypes across four diverse environments in Nigeria. The findings revealed highly significant GxE effects for grain yield, plant height, days to 50% flowering, and protein content, highlighting the strong influence of environmental factors on mungbean performance. The AMMI and GGE biplot analyses effectively captured the magnitude of these interactions, identifying Genotypes 130, 105, and 20 as the most stable and high-performing across environments. The "which-won-where" analysis delineated distinct mega-environments, demonstrating that certain genotypes exhibit specific adaptations to particular environmental conditions. Among the tested environments, UyoRain supported the highest yield and plant vigour, making it an optimal location for mungbean production. The significant GxE interactions emphasize the importance of multi-location trials in mungbean breeding programs. The identification of environment-specific and broadly adapted genotypes provides a valuable resource for developing climate-resilient mungbean varieties. Moving forward, integrating genomic selection with GxE modelling will enhance the precision of breeding strategies, ensuring the development of stable, high-yielding mungbean cultivars suitable for diverse agro-ecological zones in Nigeria.



# CHAPTER SIX

# 6.0 VARIATION IN PROTEIN COMPOSITION OF 120 MUNGBEAN GENOTYPES



## 6.1 Introduction

Mungbean protein content typically ranges from 19.1% to 33.1%, depending on various factors such as cultivar, growing conditions, and processing methods (Xia et al., 2020; Wongekalak *et al*., 2011). The protein composition of mungbean primarily consists of globulins, albumins, prolamins, and glutelins, with globulins being the most abundant (Skylas et al., 2024; Tang & Sun, 2010). This high protein content makes mungbean an essential food source, particularly in vegetarian diets prevalent in many Asian countries (Chauhan & Williams, 2018).

Variability in protein content is influenced by genetic factors, environmental conditions, and agricultural practices, highlighting the importance of selecting appropriate cultivars for maximizing nutritional yield (Kim et al., 2015). Furthermore, the protein digestibility of mungbean is enhanced through various processing techniques, such as malting and fermentation, which can also improve its functional properties (Onwurafor et al., 2020; Onwurafor et al., 2020).

Recent advancements in the processing of mungbean have also been explored to enhance its nutritional profile. Techniques such as dry fractionation have been employed to produce protein concentrates that retain high levels of protein while minimizing other constituents like carbohydrates and fats (Skylas et al., 2024; Skylas, 2025). This method not only improves the protein yield but also enhances the functional properties of mungbean protein, making it suitable for various food applications, including the development of high-protein instant noodles (Skylas, 2025).

Previous research has highlighted the importance of genetic variability in mungbean breeding (Kumar et al., 2023). However, the interaction between genotype and environment (GxE) significantly complicates breeding efforts. This study aims to dissect the relative contributions of genetic and environmental factors to protein content variation in 120 mungbean genotypes grown across four environments Awka (dry and rainy seasons) and Uyo (dry and rainy seasons).

The aim of this work is to determine the variation in protein composition of one hundred and twenty mungbean genotypes, to identify those that combine high seed yield with high seed protein content.

## 6.2 Materials and methods

### 6.2.1 Field production of mungbean seeds

The seeds of one hundred and twenty mungbean (*Vigna radiata* L. Wilczek) genotypes were analysed at the National Soil, Plant and Water Laboratory, Federal Department of Agriculture, Land and Climate Change Management Services (ALCCMS), Umudike, Abia State, Nigeria. The seeds were harvested from an experiment conducted under four environmental conditions: Awka dry season (November 2022 – February 2023), Awka rainy season (May – August 2022), Uyo dry season (December 2022 – March 2023), and Uyo rainy season (April – July 2022**)**. Each trial followed an alpha lattice design with two replications per environment. Dry-season trials were established under irrigated conditions, while rainy-season trials relied on natural precipitation. Mungbean seeds were oven-dried to a stable moisture content of approximately 12%, milled into fine powder using a hammer mill, and packaged in airtight polythene bags, clearly tagged for subsequent laboratory analysis.

### 6.2.2 Determination of moisture and protein content

#### 6.2.2.1 Determination of moisture content

Moisture content was determined according to the standard methods of Association of Official Analytical Chemists (1990). Stainless steel oven dishes were cleaned and dried in the oven at 100ºC for 1 hour to achieve a constant weight. They were cooled in a desiccator and then weighed. Two grams of sample was placed in each dish and dried in the oven at 100ºC until constant weight was achieved. The dishes together with the samples were cooled in a desiccator and weighed.

% moisture content=weight loss on drying × 100/weight of sample.

#### 6.2.2.2 Determination of Crude Protein

Crude protein was determined using the Kjeldahl method (AOAC, 1990). Two grams of sample was placed in the Kjeldahl flask. Anhydrous sodium sulphate (5g of Kjeldahl catalyst) was added to the flask. Concentrated $H_2SO_4$ (25ml) was added with few boiling chips. The flask was heated in the fume chamber until the sample solution became clear. The sample solution was allowed to cool to room temperature, then transferred into a 250ml volumetric flask and made up to volume with distilled water. The distillation unit was cleaned, and the apparatus set up. Five milliliters of 2% boric acid solution with few drops of methyl red indicator was introduced into a distillate collector (100ml conical flask). The conical flask was placed under the condenser. Then 5ml of the sample digest was pipetted into the apparatus, and washed down with distilled water. Five milliliters of 60% sodium hydroxide solution were added to the digest. The sample was heated until 100ml of distillate was collected in the receiving flask. The content of the receiving flask was titrated with 0.049M $H_2SO_4$ to a pink-coloured end point. A blank with filter paper was subjected to the same procedure.

Calculation:

$$\% \text{ Total Nitrogen} = \frac{(\text{titre} - \text{Blank}) \text{x Normality of Acid x } N_2}{\text{Weight of sample}}$$

Nitrogen factor = 6.25; Crude protein = % total N x 6.25

**6.2.3 Statistical Analysis**

Analysis of variance (ANOVA) was performed to assess the effects of genotype and environment on protein content. Statistical analyses were conducted using R statistical software (R Core Team, 2023). Significance was determined at $p < 0.05$. The ggplot2 package in RStudio (R Core Team, 2023) was used to create box plots, Histograms, Density Plots, ECDF Plots for the four environments. The AMMI analysis was conducted using the 'metan' package in R, which facilitates the analysis of multi-environment trial data (Oliveira et al., 2020). The model integrates ANOVA to assess main effects and PCA to explore interaction effects, providing a comprehensive understanding of G×E interactions.

## 6.3 Result



### 6.3.1 Variation in protein content across four environments

The protein content values generally range from approximately 22.4% to 24.1% (table 6.1). There are variations in protein content between Awka and Uyo, suggesting that location (soil, climate) influences protein accumulation. Differences between dry and rain-fed conditions indicate that water stress may affect protein content, though the direction and magnitude of this effect vary. The varying protein content across phenotypes (lines) suggests genetic differences in protein accumulation. Genotype 2 consistently shows a relatively high protein content in both Awka and Uyo under rain-fed conditions (24.09%). Genotypes 109 has a relatively low protein content (around 22.375%) across all tested conditions. Genotype 75 has a very high protein of 23.93. Genotype 84 showed the greatest difference in protein content between the conditions; with 23.34 for Awka dry protein and 22.58 for protein Uyo rain.

The minimum and maximum protein content values across all four locations indicate a relatively narrow range of variation, with values spanning from 22.10% to 24.25%. The mean protein content across locations remained consistent, ranging from 22.99% to 23.04%, suggesting that environmental effects on protein accumulation in mungbean were not highly pronounced.

However, the Coefficient of Variation (CV%) provides a clearer understanding of the relative variability in protein content. AwkarainS1 had the highest CV% (1.57%), suggesting slightly greater variability in protein content among genotypes in that environment. AwkadryS1 had the lowest CV% (1.44%), indicating more uniform performance in terms of protein content.

The standard error (SE) values were low across all locations (0.021 – 0.023), signifying that the mean protein content estimates were precise with minimal data dispersion. This reinforces the reliability of the dataset for drawing meaningful conclusions.

**Table 6.1: Statistical Summary of Seed Protein Content Across Locations**

| Statistic | AwkaDry | AwkaRain | UyoDry | UyoRain |
|---|---|---|---|---|
| Minimum | 22.10 | 22.23 | 22.35 | 22.35 |
| Maximum | 24.10 | 24.25 | 24.25 | 24.25 |
| Mean | 23.02 | 23.02 | 23.00 | 23.04 |
| Standard Error | 0.021 | 0.023 | 0.023 | 0.022 |
| CV% | 1.44% | 1.57% | 1.53% | 1.51% |

**Table 6.2: Top and bottom genotypes based on seed protein content across locations**

| Selected Genotypes | Location | | | |
|---|---|---|---|---|
| | AwkaDry | AwkaRain | UyoDry | UyoRain |
| Top five percent | 280 | 2 | 2 | 2 |
| | 111 | 280 | 280 | 75 |
| | 71 | 71 | 71 | 71 |
| | 232 | 111 | 111 | 280 |
| | 279 | 168 | 232 | 111 |
| | 129 | 232 | 15 | 232 |
| Bottom five percent | 136 | 103 | 82 | 229 |
| | 286 | 298 | 50 | 298 |
| | 298 | 164 | 164 | 164 |
| | 226 | 140 | 140 | 140 |
| | 109 | 226 | 226 | 226 |
| | 113 | 109 | 109 | 109 |

### 6.3.2 Variation in Protein Content in Environment one (Awka Dry Season)

Table 6.4 shows the ANOVA results for protein content in Awka during the dry season. The **genotype effect** was **not significant** ($F = 1.09$, $p = 0.32$), indicating that there were no substantial differences in protein content among the 120 mungbean genotypes. This suggests that environmental conditions during the dry season in Awka may have limited the expression of genetic potential for protein accumulation. Water deficit likely reduced metabolic activity and nitrogen assimilation, thereby minimizing genotypic variability. Such patterns are consistent with earlier studies showing that drought stress often suppresses expression of compositional traits in legumes due to restricted physiological processes.

**Table 6.4 Anova table for Protein Awka dry**



| SOV | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 1 | 0.08664 | 0.08664 | 0.82357678 | 0.36597056 |
| Genotype | 119 | 13.6149 | 0.11441092 | 1.08755979 | 0.32391039 |
| residual | 119 | 12.51876 | 0.10519966 | | |

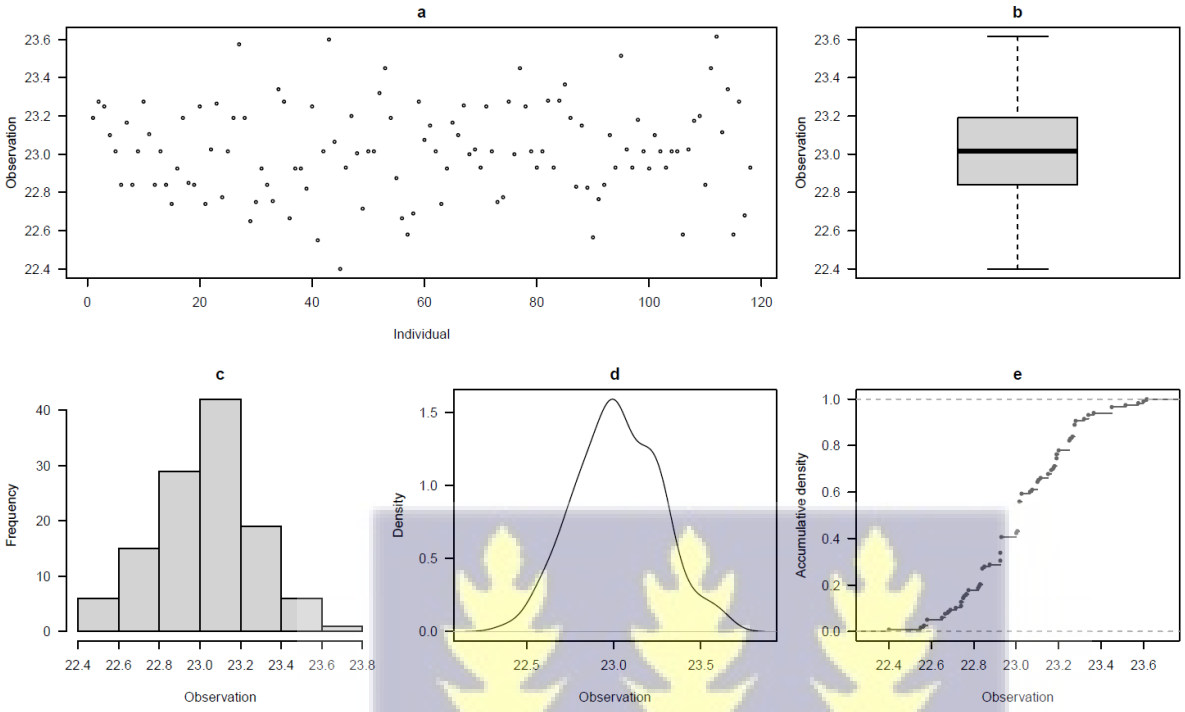


Figure 6.1 Comprehensive overview of the distribution of mung bean protein content, central tendency, and variability for environment one. [**(a) Scatter Plot:** Displaying individual data points to show their distribution over the range of observations. **(b) Box Plot (Box-and-Whisker Plot)** showing the distribution by showing the median, quartiles, and potential outliers. **(c) Histogram** showing the frequency distribution of the data by grouping observations into bins. **(d) Density Plot indicating** the probability density function, showing the distribution's shape in a smoothed form. **(e) Empirical Cumulative Distribution Function (ECDF) Plot:** Representing the cumulative proportion of observations below a particular value]

### 6.3.3 Protein Content in Environment two (Awka Rainy Season)

Table 6.5 presents the ANOVA results for Awka during the rainy season. The genotype effect was highly significant ($F = 4.38$, $p < 0.001$), showing that protein content differed significantly among genotypes under the rainy conditions in Awka. The replication effect was not significant ($F = 0.06$, $p = 0.81$). The higher moisture availability during this season likely enhanced nutrient uptake and metabolic processes, allowing genotypic differences in protein synthesis to be expressed. This suggests that adequate water availability enhances the expression of genetic potential for protein accumulation in mungbean.

**Table 6.5 ANOVA table for Protein Awka Rain**

| SOV | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 1 | 0.00273375 | 0.00273375 | 0.05629949 | 0.81285136 |
| Genotype | 119 | 25.3324796 | 0.21287798 | 4.38405904 | 5.898E-15 |
| Residual | 119 | 5.77831625 | 0.04855728 | | |



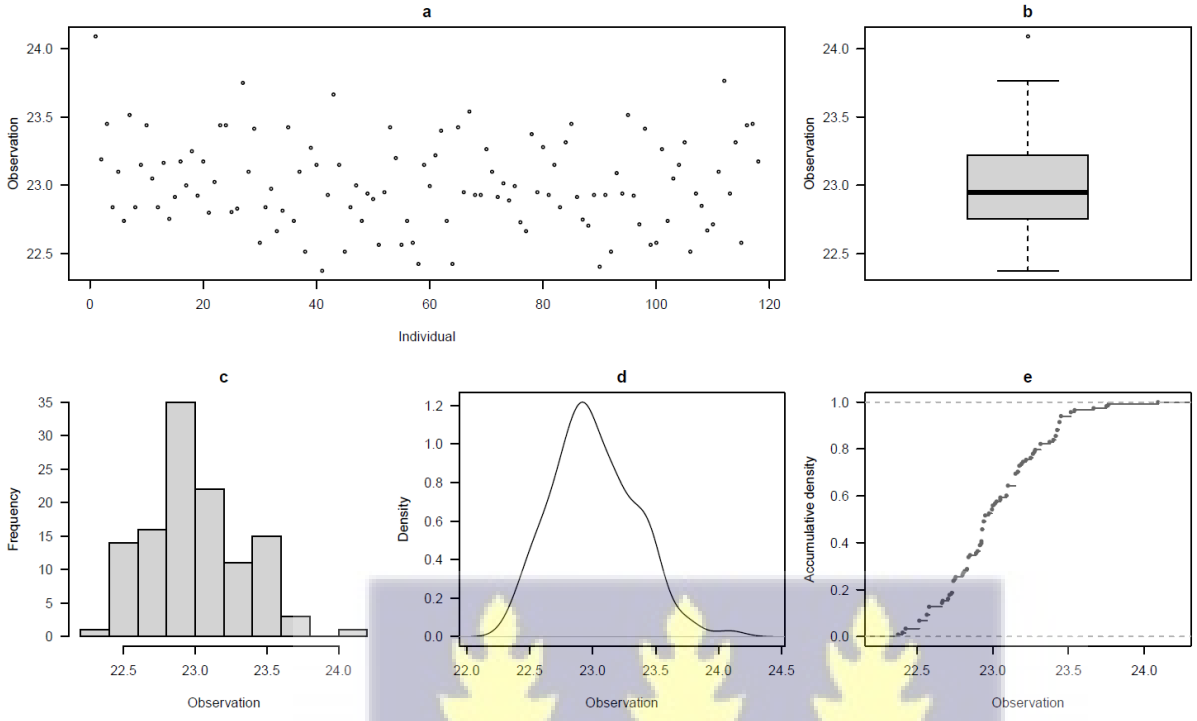


**Figure 6.2** Comprehensive overview of the distribution of mung bean protein content, central tendency, and variability for environment two. [**(a) Scatter Plot:** Displaying individual data points to show their distribution over the range of observations. **(b) Box Plot (Box-and-Whisker Plot)** showing the distribution by showing the median, quartiles, and potential outliers. **(c) Histogram** showing the frequency distribution of the data by grouping observations into bins. **(d) Density Plot indicating** the probability density function, showing the distribution's shape in a smoothed form. **(e) Empirical Cumulative Distribution Function (ECDF) Plot:** Representing the cumulative proportion of observations below a particular value]

### 6.3.4 Protein Content in Environment three (Uyo Dry Season)

Table 6.6 shows the ANOVA results for Uyo during the dry season. The genotype effect was highly significant ($F = 5.52$, $p < 0.001$), indicating considerable genetic variation among the mungbean genotypes in this environment. Although replication was not significant ($F = 1.37$, $p = 0.24$), the significant genotype effect suggests that genetic differences influenced protein accumulation even under dry conditions. This observation implies that while environmental stress can reduce protein synthesis, certain genotypes maintained superior performance, possibly due to inherent tolerance to water stress and efficient nitrogen metabolism.

Table 6.6 ANOVA table for Protein Uyo Dry

| SOV | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 1 | 0.052215 | 0.052215 | 1.36896727 | 0.24432737 |
| Genotype | 119 | 25.0642983 | 0.21062436 | 5.52212676 | 6.0681E-19 |
| Residual | 119 | 4.538885 | 0.03814189 | | |

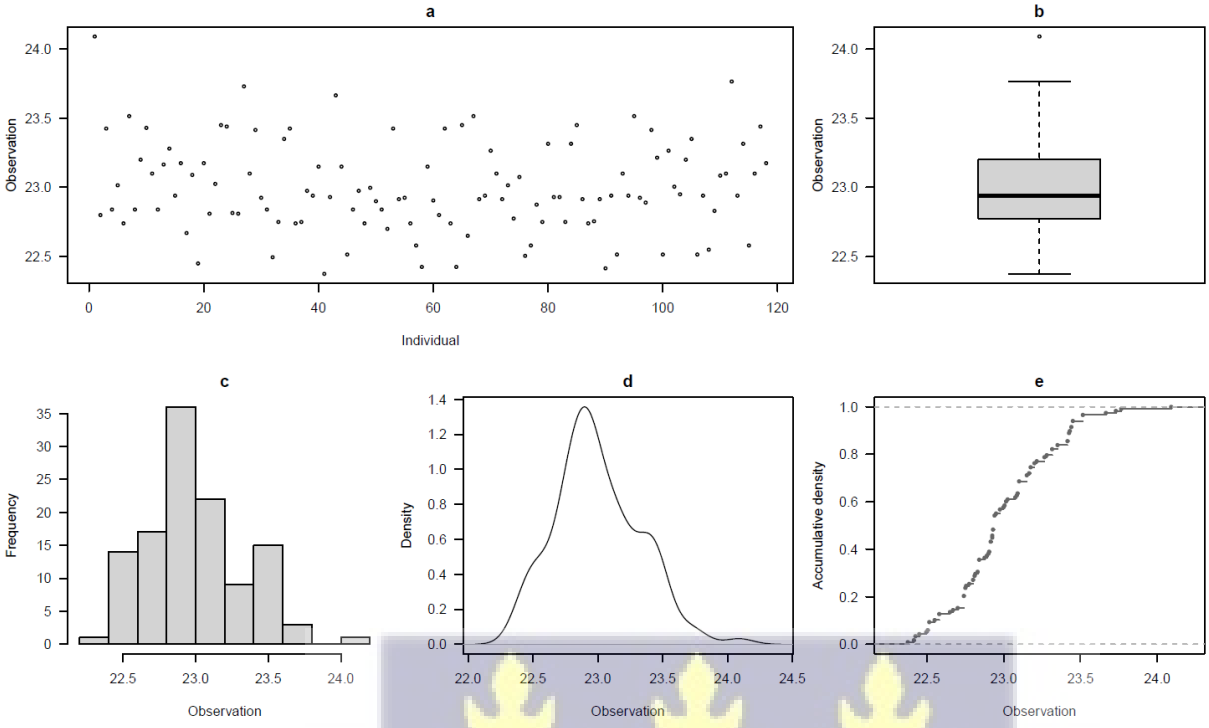


Figure 6.3 Comprehensive overview of the distribution of mung bean protein content, central tendency, and variability for environment three [**(a) Scatter Plot:** Displaying individual data points to show their distribution over the range of observations. **(b) Box Plot (Box-and-Whisker Plot)** showing the distribution by showing the median, quartiles, and potential outliers. **(c) Histogram** showing the frequency distribution of the data by grouping observations into bins. **(d) Density Plot indicating** the probability density function, showing the distribution's shape in a smoothed form. **(e) Empirical Cumulative Distribution Function (ECDF) Plot:** Representing the cumulative proportion of observations below a particular value]

### 6.3.5 Protein Content in Environment four (Uyo Rainy Season)

Table 6.7 presents the ANOVA results for Uyo during the rainy season. The genotype effect was highly significant ($F = 6.74$, $p < 0.001$), indicating that the genotypes differed widely in their protein content under the humid Uyo conditions. Replication was not significant ($F = 0.73$, $p = 0.39$). The increased rainfall and soil moisture likely enhanced nitrogen uptake and assimilation, leading to greater protein biosynthesis and clearer expression of genetic variability. These findings align with earlier studies reporting that moisture availability enhances protein content in legumes due to improved nutrient solubility and root absorption.

**Table 6.7 ANOVA table for Protein Uyo Rain**

| SOV | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Rep | 1 | 0.022815 | 0.022815 | 0.73298345 | 0.39365306 |
| Genotype | 119 | 25.1684317 | 0.20973693 | 6.73827307 | 1.4378E-22 |
| residual | 119 | 3.67289328 | 0.03112621 | | |



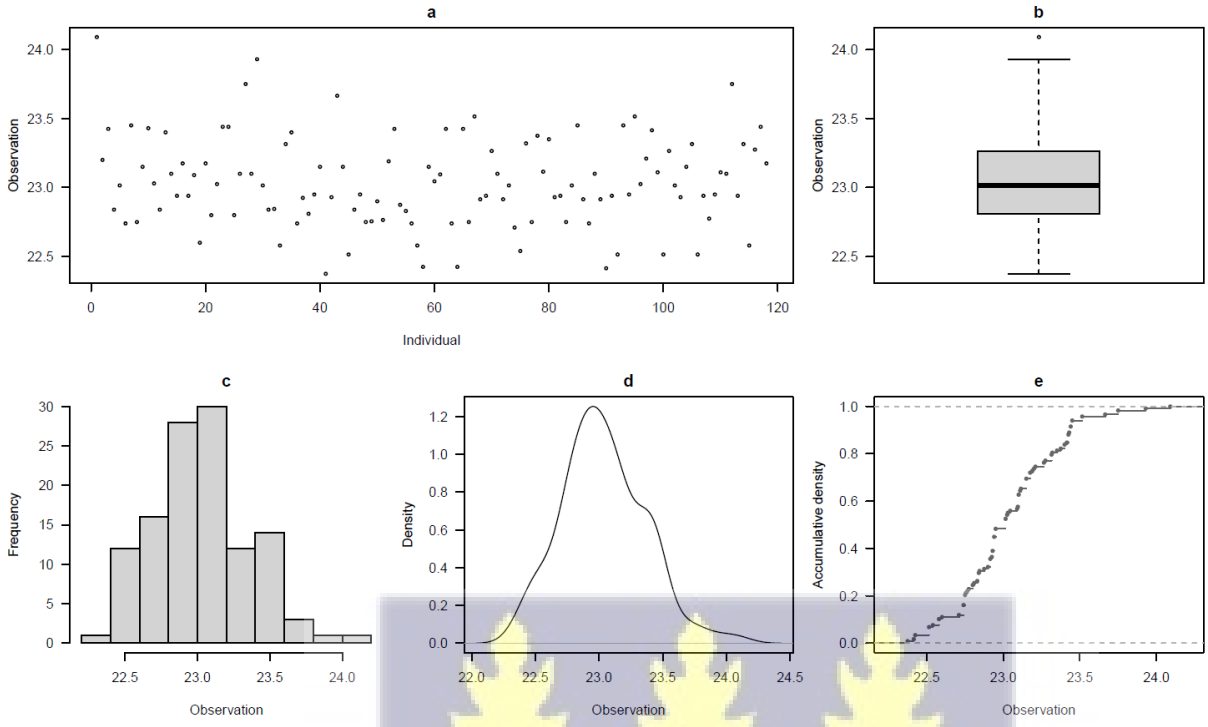


**Figure 6.4** Comprehensive overview of the distribution of mung bean protein content, central tendency, and variability for environment four. [**(a) Scatter Plot:** Displaying individual data points to show their distribution over the range of observations. **(b) Box Plot (Box-and-Whisker Plot)** showing the distribution by showing the median, quartiles, and potential outliers. **(c) Histogram** showing the frequency distribution of the data by grouping observations into bins. **(d) Density Plot indicating** the probability density function, showing the distribution's shape in a smoothed form. **(e) Empirical Cumulative Distribution Function (ECDF) Plot:** Representing the cumulative proportion of observations below a particular value]

## 6.4 AMMI ANOVA showing the GXE interaction

The AMMI (Additive Main Effects and Multiplicative Interaction) analysis revealed a highly significant genotype effect ($F = 10.61$, $p < 2.39 \times 10^{-80}$), indicating substantial genetic variation among the 120 mungbean genotypes for seed protein content (Table 6.8). However, the environmental effect was not significant ($F = 1.31$, $p = 0.27$), suggesting that mean differences among the four test environments (Awka dry, Awka rainy, Uyo dry, and Uyo rainy) were minimal. The G×E interaction was also not significant ($F = 0.96$, $p = 0.66$), implying that genotypes responded relatively consistently across environments. Nonetheless, when

decomposed into interaction principal components, the first principal component (IPC1) captured a substantial portion of the G×E variation (Sum of Squares = 36.82; F = 5.43; $p < 1.11 \times 10^{-16}$), indicating meaningful differential genotype responses under certain environmental conditions**.** IPC2 and IPC3 were not significant ($p > 0.99$), confirming that most of the interaction was explained by IPC1. Among the genotypes, G50 and G85 showed broad adaptability and high protein content, particularly in rain-fed environments. The significant IPC1 thus reflects structured environmental influence primarily linked to water availability that interacts with genotype performance.

Table 6.8 AMMI ANOVA for Protein content for 120 mungbean genotypes

| Source | Sum of Squares | Df | Mean Square | F-Value | P-Value |
|---|---|---|---|---|---|
| Genotype (GEN) | 70.16 | 119 | 0.59 | 10.609 | $2.39 \times 10^{-80}$ |
| Environment (ENV) | 0.22 | 3 | 0.07 | 1.310 | $2.70 \times 10^{-1}$ |
| GEN × ENV | 19.02 | 357 | 0.05 | 0.959 | $6.62 \times 10^{-1}$ |
| IPC1 | 36.82 | 122 | 0.30 | 5.432 | $1.11 \times 10^{-16}$ |
| IPC2 | 4.19 | 121 | 0.03 | 0.623 | 0.999 |
| IPC3 | 1.96 | 120 | 0.02 | 0.294 | 1.000 |
| Residual | 26.67 | 480 | 0.06 | - | - |

## 6.5 Discussion

The findings of this study highlight the predominant influence of environmental factors, particularly water availability, on seed protein content in mungbean (*Vigna radiata* [L.] Wilczek). The generally non-significant genotype effects observed within individual environments (Awka dry, Awka rainy, Uyo dry, and Uyo rainy seasons) indicate that environmental variation largely overshadowed the expression of genetic potential for protein accumulation. This suggests that protein synthesis in mungbean is highly responsive to moisture conditions and other environmental cues, emphasizing the importance of site-specific management practices in achieving optimal nutritional outcomes. Although genotype effects were not significant within environments, the AMMI analysis revealed highly significant genotype effects ($p < 2.39 \times 10^{-80}$) across environments, indicating the presence of inherent genetic variation. The first interaction principal component (IPC1) captured a substantial proportion of the genotype × environment (G×E) variation ($p < 1.11 \times 10^{-16}$), suggesting that while environmental effects dominate, certain genotypes respond differently to specific environmental conditions. Notably, genotypes 50 and 85 exhibited adaptability and stable protein performance

across the rain-fed environments (E2 and E4). This pattern is consistent with the report of Maphosa *et al*. (2023), who demonstrated that environmental heterogeneity significantly contributes to phenotypic variation in plant traits.



Our results align with earlier studies in legumes that have documented strong environmental influences on seed protein content. Dornbos and Mullen (1991) reported up to a 20% reduction in soybean seed protein under drought conditions, while Beebe *et al*. (2013) demonstrated that temperature and water availability were key determinants of protein accumulation in common bean. Similarly, in chickpea, irrigation was shown to enhance seed protein content through improved nitrogen uptake and assimilation (Gowda *et al*., 2009). The significant environmental effects observed in the rainy-season environments of Awka and Uyo in this study likely reflect enhanced nitrogen uptake under adequate soil moisture (Dahiya *et al*., 2015), as nitrogen is a vital component of amino acids and proteins (Marschner, 2012). Differences in protein content between Awka and Uyo further suggest the influence of soil fertility and microclimatic variation. Imtiaz (2003) earlier observed that soil nutrient composition significantly affects mungbean protein levels, emphasizing the interaction of edaphic and climatic factors in shaping nutrient accumulation. Hence, local soil testing and fertility management could help identify limiting factors that restrict protein synthesis under specific field conditions. The apparent lack of significant genotype effects within individual environments does not imply genetic uniformity. Instead, it suggests that the environmental range under study may not have been broad enough to fully reveal genotypic differences. Under optimal or more contrasting conditions, these genetic variations may become more apparent. Previous studies have reported substantial genetic diversity for seed composition traits in mungbean (Keatinge *et al*., 2011; Upadhyaya *et al*., 2011; Kumar *et al*., 2023). Therefore, expanding the evaluation to include more diverse germplasm and multi-environment testing would help to identify stable, high-protein genotypes. From a breeding perspective, the significant genotype × environment interaction detected through IPC1 underscores the need for stability analysis in selection programs. GGE biplot or AMMI-based models can effectively identify genotypes that combine high mean protein content with stability across diverse environments (Yan & Kang, 2003). Integrating molecular tools such as marker-assisted

selection and genomic prediction could accelerate the identification of alleles associated with protein synthesis and nitrogen metabolism (Upadhyaya et al., 2011).



The study demonstrates that while environmental conditions particularly water availability exert the strongest influence on mungbean protein content, considerable genetic variability exists that can be harnessed through multi-environment and molecular breeding approaches. Future research should combine field phenotyping with molecular characterization to elucidate the genetic basis of protein stability across environments. Additionally, postharvest processing techniques such as fermentation and germination, which improve nutrient bioavailability (Onwurafor et al., 2020), could be explored to enhance the nutritional quality and utilization of mungbean as a dietary protein source.

### 6.6 Conclusion

The present study revealed that both genetic and environmental factors play crucial roles in determining seed protein content in mungbean (*Vigna radiata* [L.] Wilczek). Although environmental variability particularly moisture availability exerted the strongest influence on protein accumulation, the AMMI analysis confirmed significant genetic variation among genotypes, suggesting that heritable differences exist that can be exploited in breeding programs. The identification of stable genotypes such as 50 and 85, which demonstrated consistent protein performance across rain-fed environments, underscores their potential as candidates for varietal improvement and dissemination in regions with variable rainfall patterns. The relatively low genotype × environment (G×E) interaction indicates that most genotypes responded consistently across locations, highlighting the feasibility of selecting broadly adapted lines for protein quality. However, the significant contribution of the first interaction principal component (IPC1) suggests that specific environmental factors, notably water availability and soil fertility, still interact with genotype performance to a measurable extent. These findings provide a valuable foundation for future breeding efforts aimed at enhancing the nutritional quality and stability of mungbean under diverse agro-ecological conditions.

# 7.0 Genome Wide Association Studies of Mungbean for Yield and Protein Content

## 7.1 Introduction

Mungbean plays a significant role in sustainable agriculture due to its ability to fix atmospheric nitrogen, thus enhancing soil fertility and contributing to intercropping systems (Khan, 2023; Khan *et al*., 2017). The yield and protein content of mungbean are critical traits that influence its economic viability and nutritional benefits. Recent studies have highlighted the importance of optimizing these traits through genetic improvement and breeding strategies (Hannan *et al*., 2022; Doutaniya *et al*., 2023).

Mungbean is a significant legume crop in Nigeria, contributing to food security and nutritional needs due to its high protein content and adaptability to various agro-ecological conditions. Recent advancements in genome-wide association studies (GWAS) have provided insights into the genetic basis of important traits such as yield and protein content, which are crucial for enhancing mungbean production in Nigeria.

In Nigeria, the cultivation of mungbean has been gaining attention as a viable source of protein, particularly in regions where other legumes may not thrive due to climatic constraints. The genetic diversity present in mungbean germplasm is essential for breeding programs aimed at improving yield and nutritional quality. For instance, Akhtar *et al*. (2021) conducted a GWAS focusing on seed weight, a critical trait influencing yield. Their findings highlighted specific quantitative trait loci (QTLs) associated with this trait, which can be leveraged in breeding programs to select for higher yielding varieties. This is particularly relevant for Nigerian farmers who often face challenges related to low yield and poor seed quality.

Moreover, the study by Reddy *et al*. (2021) on phosphorus use efficiency traits in mungbean is particularly pertinent to Nigeria, where soil fertility can be a limiting factor in crop production. Their research identified several SNPs associated with phosphorus uptake and utilization efficiency, which can be crucial for developing mungbean varieties that are better adapted to the nutrient-poor soils commonly found in many Nigerian agricultural zones. This genetic insight can facilitate the selection of mungbean varieties that not only yield better but also require fewer inputs, thereby promoting sustainable agricultural practices.

Genetic diversity is fundamental for the successful breeding of mungbean, as it allows for the selection of superior genotypes with desirable traits. The use of molecular markers, particularly single nucleotide polymorphisms (SNPs), has revolutionized the approach to genetic mapping and association studies in crops (Shaibu *et al*., 2021; Adeboye *et al*., 2020). The Diversity Array Technology (DArT) combined with SNP data provides a robust framework for assessing genetic variation and conducting genome-wide association studies (GWAS) (Muchira *et al*., 2021). The mrMLM model, a statistical method for GWAS, is particularly effective in identifying genetic loci associated with complex traits such as yield and protein content (Adeboye *et al*., 2020; Zhang *et al*., 2021).

The application of the mrMLM model in conjunction with DArTseq SNP data not only enhances the resolution of GWAS but also facilitates the identification of epistatic interactions among loci that may influence complex traits. This is particularly important in mungbean, where traits such as yield and protein content are polygenic and influenced by multiple environmental factors. Understanding these interactions can lead to more effective breeding strategies that consider the multifaceted nature of trait expression in mungbean.

In addition to yield, protein content is a vital trait for improving the nutritional value of mungbean. Liu *et al.* (2022) provided a comprehensive analysis of seed-size-related traits, which are often correlated with protein content. Their findings suggest that genetic markers associated with these traits can be utilized to enhance the protein quality of mungbean, making it a more valuable food source in Nigeria, where protein malnutrition is a significant concern. The ability to identify and select for these traits through GWAS can lead to the development of mungbean varieties that meet both agronomic and nutritional needs.

Furthermore, the work of Manjunatha (2024) on various agro-economical traits in mungbean emphasizes the importance of understanding the genetic control of traits such as flowering time and plant height. These traits are crucial for optimizing mungbean cultivation in Nigeria's diverse climatic conditions. By identifying candidate genes associated with these traits, breeders can develop varieties that are not only high-yielding but also resilient to the changing climate, which is increasingly affecting agricultural productivity in the region.

The integration of GWAS findings into breeding programs in Nigeria can significantly enhance the efficiency of mungbean production. For example, the research by Han *et al.* (2022) on genetic loci associated with key agronomic traits provides a framework for selecting superior genotypes that can thrive in Nigerian conditions. This approach aligns with the broader goals of improving food security and nutritional quality in the country.

The application of genome-wide association studies in mungbean research presents a promising avenue for enhancing both yield and protein content in Nigeria. By leveraging the genetic insights gained from these studies, Nigerian plant breeders can develop improved mungbean varieties that are better suited to local conditions, ultimately contributing to the country's food security and nutritional needs.

The identification of quantitative trait loci (QTLs) associated with protein content and yield traits will establish a foundation for developing improved mungbean varieties capable of enhancing food security and nutritional quality in the region. As the demand for high-quality plant-based protein sources continues to increase, the insights derived from this study will be invaluable to breeders and agricultural stakeholders seeking to optimize mungbean productivity and adaptability in Nigeria. Therefore, the aim of this study was to identify genomic regions and molecular markers linked to protein content and yield-related traits in mungbean through genome-wide association studies (GWAS), to support marker-assisted breeding for nutritional improvement and yield stability.

## 7.2 Materials and Methods

### 7.2.1 Experimental Sites and Description

The study was conducted at four distinct environments representing two agro-ecological zones in southern Nigeria: Awka (Anambra State) and Uyo (Akwa Ibom State), each evaluated under rainy and dry season conditions. Awka (6°12′ N, 7°04′ E; 137 m a.s.l.) lies within the humid tropical rainforest zone, with mean annual rainfall ranging between 1,800 and 2,200 mm and mean daily temperatures from 26 °C to 33 °C. Uyo (5°03′ N, 7°56′ E; 65 m a.s.l.) has a similar humid tropical climate, receiving between 2,000 and 2,500 mm of rainfall annually. The experiments were carried out during the Awka dry season (November 2022 – February 2023), Awka rainy season (May – August 2023), Uyo dry season (December 2022 – March 2023), and Uyo

rainy season (April – July 2023). Dry-season trials were conducted under supplementary irrigation, while rainy-season trials relied on natural precipitation. A total of 120 mungbean (Vigna radiata L. Wilczek) genotypes obtained from the World Vegetable Center minicore collection were evaluated. These accessions represent a diverse genetic base of introduced germplasm being assessed for adaptability and agronomic performance under Nigerian growing conditions. Prior to sowing, seeds were oven-dried to approximately 12 % moisture content, surface-sterilized, and treated with thiram (2 g $kg^{-1}$) to prevent fungal infection.

Each trial was laid out in an alpha-lattice design (10 × 12) with two replications per environment to minimize experimental error and account for field heterogeneity. Each genotype was planted in a two-row plot measuring 4 m × 1 m, with row spacing of 50 cm and intra-row spacing of 10 cm, accommodating approximately 40 plants per plot. Standard agronomic practices were followed across sites: manual weeding at 3 and 6 weeks after sowing, pest management with cypermethrin at recommended rates, and no chemical fertilizer application to allow genotypic performance under moderate soil fertility.

### 7.2.2 Genotyping and Quality Assessment

SNP data were generated using DArT-seq technology, which provides a high-throughput method for genotyping and is particularly useful for crops with limited genomic resources (Shaibu *et al.,* 2021; Muchira et al., 2021). The DArT-seq markers were selected based on their polymorphism information content (PIC), ensuring that only informative markers were included in the analysis (Adeboye *et al*., 2020). The mrMLM model was employed to perform GWAS, allowing for the identification of significant SNPs associated with yield and protein content. This model accounts for population structure and kinship, which are critical factors in plant breeding studies (Zhang *et al.,* 2021; Milczarski *et al.,* 2011).

The process of DNA extraction and SNP discovery and quality assessment have been discussed extensively in chapter four

### 7.2.3 Determination of Protein content:



Protein content was determined using the Kjeldahl method, a widely accepted technique for protein analysis in legumes (Hannan et al., 2022; Doutaniya et al., 2023). Two grams of sample was placed in the Kjeldahl flask. Anhydrous sodium sulphate (5g of Kjeldahl catalyst) was added to the flask. Concentrated $H_2SO_4$ (25ml) was added with few boiling chips. The flask was heated in the fume chamber until the sample solution became clear. The sample solution was allowed to cool to room temperature, then transferred into a 250ml volumetric flask and made up to volume with distilled water. The distillation unit was cleaned, and the apparatus set up. Five milliliters of 2% boric acid solution with few drops of methyl red indicator was introduced into a distillate collector (100ml conical flask). The conical flask was placed under the condenser. Then 5ml of the sample digest was pipetted into the ap

paratus, and washed down with distilled water. Five milliliters of 60% sodium hydroxide solution were added to the digest. The sample was heated until 100ml of distillate was collected in the receiving flask. The content of the receiving flask was titrated with 0.049M $H_2SO_4$ to a pink-coloured end point. A blank with filter paper was subjected to the same procedure.

Calculation:

$$\% \text{ Total Nitrogen} = \frac{(\text{titre} - \text{Blank}) \text{x Normality of Acid x } N_2}{\text{Weight of sample}}$$

Nitrogen factor = 6.25; Crude protein = % total N x 6.25

Determination of Yield:

Yield was quantified by harvesting the plants and calculating the total biomass; estimation of grain yield and 100-seed weight: 100 seeds were manually counted and weighed in triplicate. Grain yield was recorded in grams per plot in individual replicates and converted to kg ha−1. Border rows were excluded from the plot yield estimation to eliminate border effects.

### 7.2.4 **Statistical Analysis**

#### 7.2.4.1 Phenotypic data analysis

The "augmentedRCBD" function from the R package "agricolae" for the DAU test function was used to assess the phenotypic data gathered from the four environments (De Mendiburu, 2015). Blocks and treatments were regarded as random in the model, whereas checks were regarded as fixed. Using mixed model analysis as shown in the equation, the adjusted means for the different genotypes in the two cropping seasons were then used in GWAS analysis (R Core Team, 2022).

Where;

- **Y:** The phenotypic trait value.
- **$\beta_0$:** The intercept of the model.
- **$\beta_i$:** The effect size (regression coefficient) of the ith SNP genotype (typically coded as 0, 1, or 2 for the number of minor alleles).
- **Xi:** The genotype score for the ith SNP.
- **$\gamma j$:** The effect size of the jth covariate.
- **Cj:** The value of the jth covariate for each individual (polulation structure).
- **$\varepsilon$:** The error term, representing all other unmeasured factors influencing the trait.

Statistical analyses were conducted to evaluate the significance of the associations identified through GWAS, providing insights into the genetic basis of these important traits.

#### 7.2.4.1 Genome Wide Association Study Analysis and Gene Identification

Five genetic models were used to calculate the relationships using a mixed linear model that was implemented in the multi-random mixed linear model (mrMLM) (Zhang *et al.*, 2017). These models included the following: polygenic-background-control-based least angle regression plus empirical Bayes (pLARmEB) (Zhang *et al.*, 2017), fast multi-locus random-SNP-effect Mixed Linear Model (Wang *et al.*, 2016), fast multi-locus random-SNP-effect EMMA (FASTmrEMMA) (Zhang *et al.*, 2020), fast mrMLM (FASTmrMLM) (Tamba and Zhang, 2018) and pKWmEB (Wen *et al.*, 2018). The effectiveness of the GWAS model in compensating for population structure was evaluated by plotting the observed logarithms (-log10) of the p-values versus the anticipated p-values.

After finding a significant SNP marker, zoom mapping was done on a certain chromosome and the Manhattan plot was made to visualize GWAS on the entire genome. We used the general feature format (GFF3) to look for genes in the closest related marker in order to identify the genes (Hunter *et al.,* 2012). The functions of the genes linked to the various SNPs found were ascertained using the public database Interpro, European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) (Shin *et al.,* 2006).

### 7.3 Result

#### 7.3.1 Genetic Relationship Among Mungbean Genotypes Based on PCA Analysis

Principal Component Analysis (PCA) was performed to explore the genetic relationship among mungbean genotypes using SNP markers. The PCA biplot (Figure 7.1) revealed distinct clustering patterns, suggesting varying degrees of genetic relatedness among the genotypes. The first two principal components (PC1 and PC2) accounted for a substantial proportion of the total genetic variation, indicating that these axes effectively capture the primary structure of genetic diversity within the dataset.

The clustering pattern observed in the PCA biplot suggests that some genotypes share close genetic ancestry, as evidenced by their proximity in the plot. In contrast, genotypes that are more dispersed exhibit greater genetic divergence. This genetic variation could be attributed to factors such as selection pressure, domestication history, or geographical adaptation. The presence of well-defined clusters may indicate distinct genetic subpopulations within the mungbean accessions, which could be useful for breeding programs aiming to enhance genetic heterozygosity.

The results align with previous studies on mungbean genetic diversity, which have shown that accessions from different geographical regions or breeding backgrounds tend to cluster separately in multivariate analyses (Sudha et al., 2013; Gwag et al., 2010). The findings provide valuable insights for plant breeders in selecting diverse parental lines for hybridization to maximize heterosis and genetic gain.

#### 7.3.2 Linkage Disequilibrium (LD) Patterns in the Mungbean Genome

The linkage disequilibrium (LD) heatmap (Figure7.2) illustrates the correlation between SNP markers across the mungbean genome. LD, measured as the squared correlation coefficient ($r^2$), serves as an indicator of how

SNPs are inherited together over generations. The observed LD pattern reveals both high and low LD regions, suggesting variable recombination rates across different genomic regions.



High LD blocks indicate genomic regions where SNPs are strongly associated, potentially due to low recombination rates, recent selection, or population bottlenecks. These regions may harbor functionally important genes that have been subject to selection during mungbean domestication or breeding. Conversely, regions of low LD suggest frequent recombination events, leading to a breakdown of SNP associations over time.

The LD decay pattern is particularly important for genome-wide association studies (GWAS) and marker-assisted selection (MAS) in mungbean breeding programs. Faster LD decay implies a higher resolution for genetic mapping, whereas slow LD decay suggests larger haplotype blocks, which may require denser marker coverage for precise association mapping. The observed LD structure is consistent with previous reports in other legumes, such as soybean and cowpea, where LD varies across chromosomes due to differences in recombination hotspots and genomic architecture (Huang et al., 2016; Khaing et al., 2022)

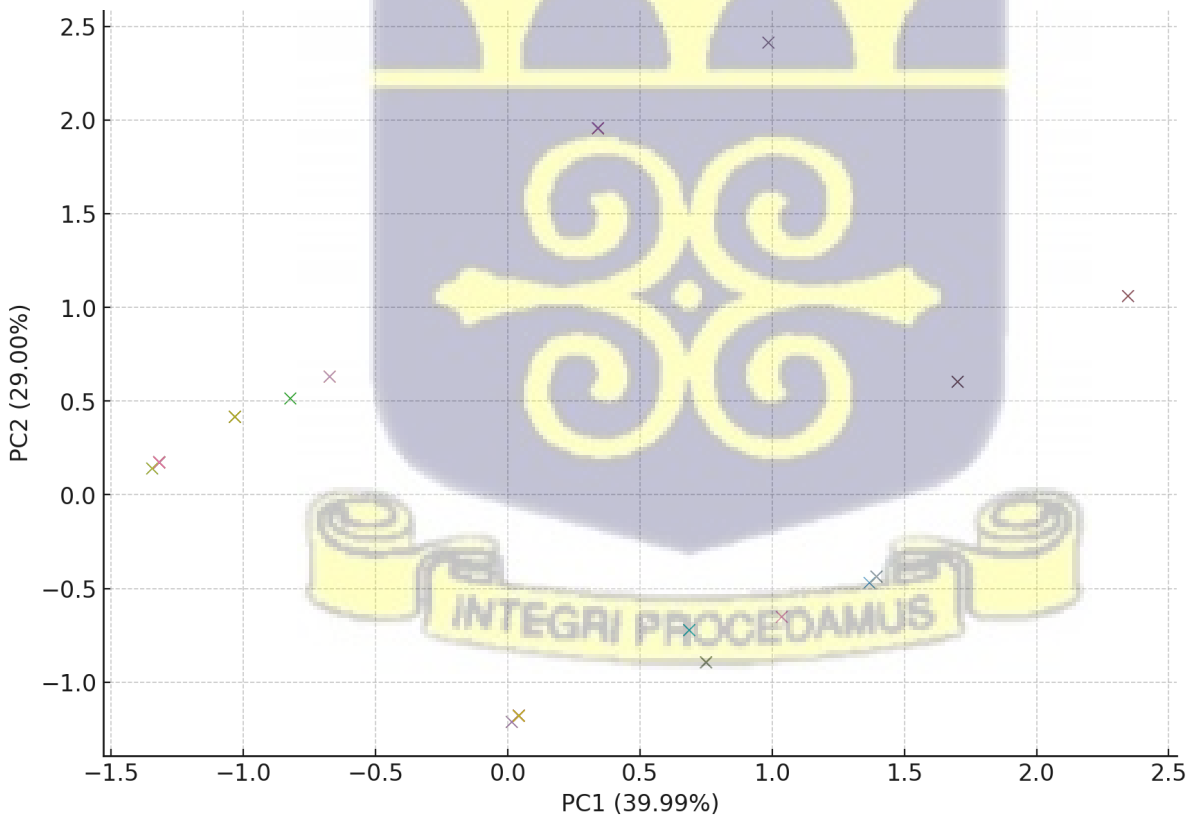


Fig 7.1 PCA-biplot based clustering displaying the relationship between and among mungbean genotypes

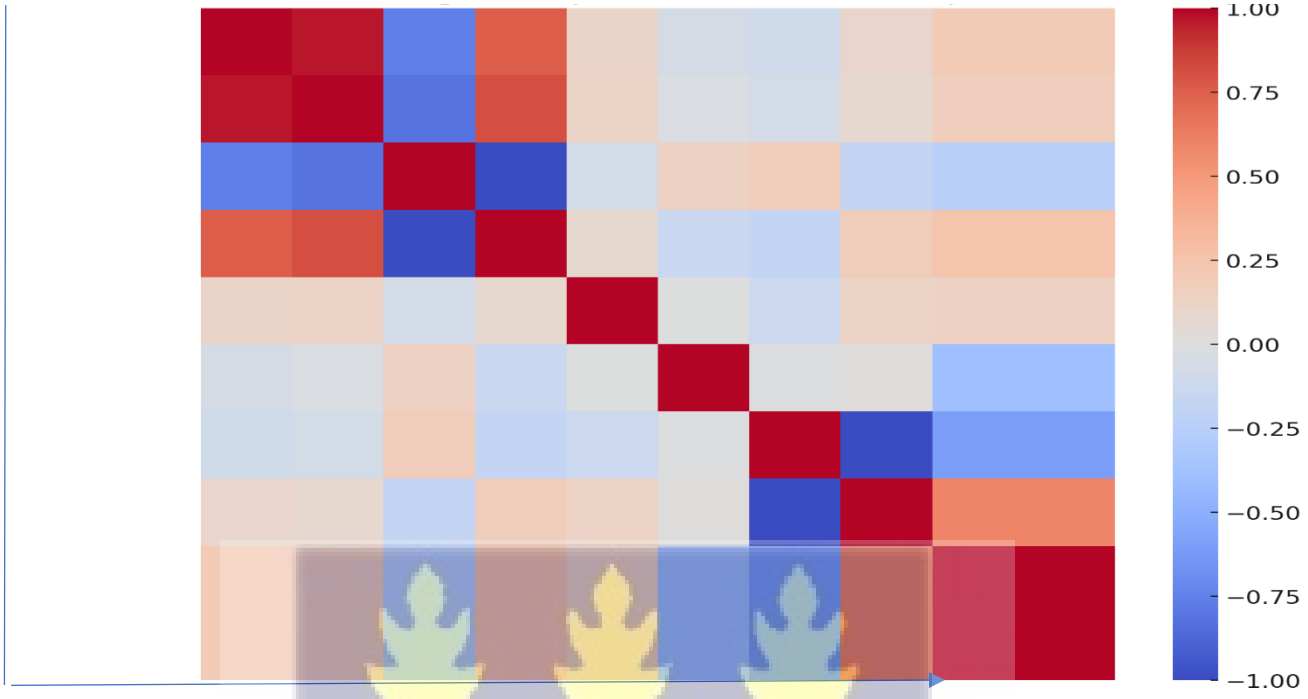


Fig 7.2 Linkage disequilibrium (LD) heatmap of mungbean genome used for the study

### 7.3.3 Genes Influencing Protein Content

In Environment One (Awkadry), the most significant marker associated with protein content was Chr1_2908989, identified by the mrMLM method (Table 7.1 and 7.2). This marker is located within the region of the *EC_6.2 ligase forming carbon-sulfur* gene, which had a minor allele frequency (MAF) of 0.4831 and explained 12.71% of the phenotypic variance. These findings suggest that this locus is a major contributor to protein content in this environment. Additional markers identified with FASTmrMLM, FASTmrEMMA are also linked to the same gene (Chr1_2908989), further supporting the importance of the locus. *The function of the identified markers, chr3_7994659 identified using mrMLM, FASTmrEMMA is Resection MRE11-RAD50-NBS1 (MRN). The marker, Chr1_12963638 identified with pLARmEB had LRK91_ARATH (Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK)) gene function*. In Environment Two (Awkarain), the pLARmEB method identified Chr5_3185830 as significantly associated with protein content, with a MAF of 0.3167 and

explaining 2.84% of the phenotypic variance. This marker is located near an *F-Box Protein (FB49_ARATH)* gene. The function of the identified marker, Chr2_809453 identified using pLARmEB is SRS1(SHI related sequence 1) SRS-type transcription factor, similarly, the function of the identified marker, Chr5_25318711 identified using pLARmEB is Transcription Elongation Factor B Polypeptide. In Environment Three (Uyodry), the pLARmEB method identified chr6_569503 as significantly associated with protein content, located within C2H2 Zinc Finger Transcription factor. In Environment Four (Uyorain), the marker associated with protein content was chr5_3185830 near *FB49_ARATH* (regulates protein degradation through the ubiquitin-proteasome system (UPS). The identification of multiple loci associated with protein content across different environments highlights the complex genetic architecture of this trait

### 7.3.4 Genes Influencing Yield

In Environment One (Awkadry), the marker associated with yield was chr3_9213031. Several methods (mrMLM, FASTmrMLM, pLARmEB, and ISIS EM-BLASSO) identified this marker, which is located near an *EC_2.1 transferase transferring one-carbon* gene. *The function of the chr4_19961226 identified using FASTmrMLM, pLARmEB, ISIS EM-BLASSO is Unclassified protein.* In Environment Two (Awka rain), the markers associated with Yield were chr3_6148955, chr5_15655284, chr6_29354314. These were located on *EGC_CITJA; XP_014504277.1; PNP precursor polypeptide*, *embryogenesis abundant protein; XP_014517278.1*, and *Microtubule-associated protein crucial for spindle assembly during cell division spF4I2H7|TPX2_ARATH; XP_014493011.1*. In Environment Three (Uyodry), the markers associated with Yield were chr2_21263131, chr2_2956559, chr4_19860552, chr5_16189831, chr8_42327370. These were located on *Uncharacterized protein*, *BKI1 (Brassinosteroid Kinase Inhibitor 1)*, *Uncharacterized protein*, *Fasciclin-Type Arabinogalactan Protein (FLA1)*, and *Unknown*. In Environment four (Uyorain), the markers associated with Yield were chr5_5871534, chr6_30896900, chr8_44276921, chr9_10783673. These were located on *Belongs to the AAA ATPase family; CDC48_SOYBN; XP_014518955.1*, *THYLAKOID FORMATION1; regulatory protein (THF1) of thylakoid*, *Transcription elongation factor B polypeptide*, and *mitochondrial carrier family; EAAC_ARATH; solute transporter*. The identification of these MTAs provides

valuable insights into the genetic architecture of yield in mungbean and can be used to guide marker-assisted selection in breeding programs (Du *et al*., 2025).



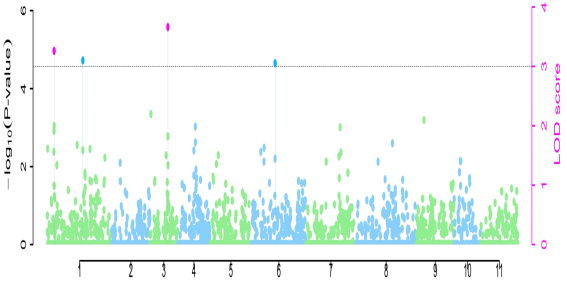


Fig7.3a Manhattan Plot ENV1-Awka dry Protein

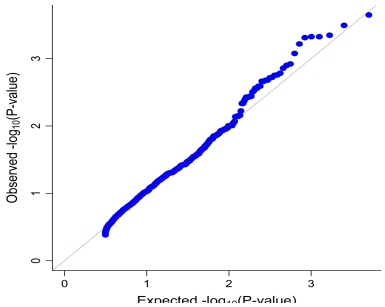


Fig7.3b QQ Plot ENV1-Awka dry

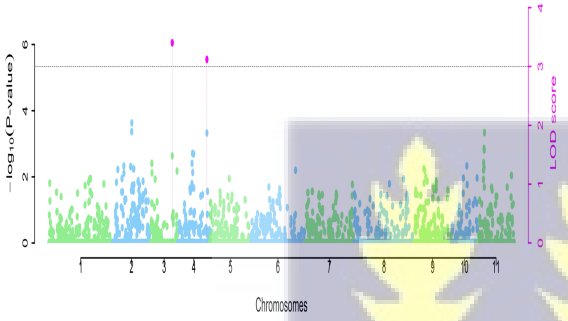


Fig7.4a. Manhattan Plot ENV1-Awka dry Yield

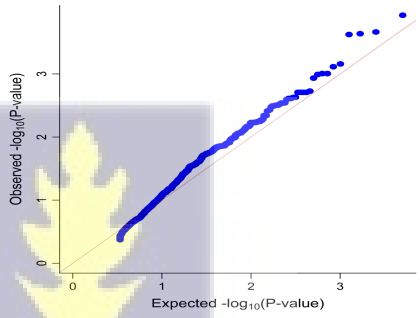


Fig7.4b. QQ Plot ENV1-Awka dry Yield

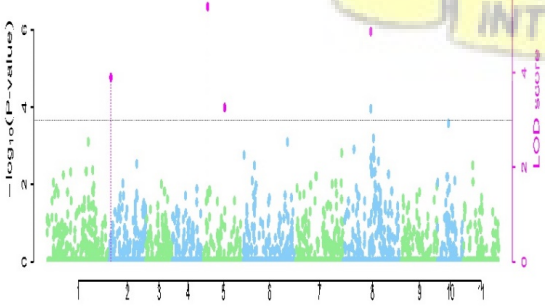


Fig 7.5a: Manhattan Plot ENV2-Awka Rain Protein

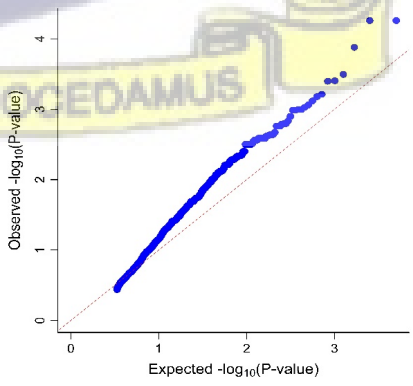

Fig 7.5b: QQ Plot ENV2-Awka Rain Protein

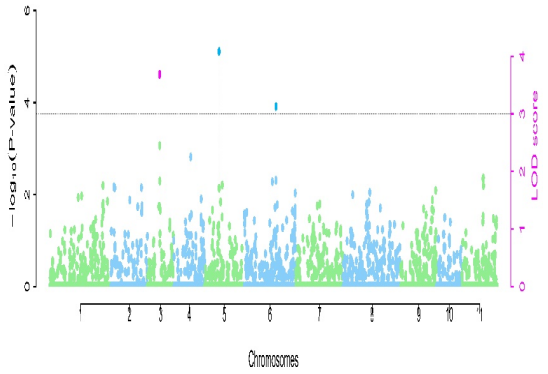


Fig 7.6a: Manhattan Plot ENV2-Awka Rain Yield

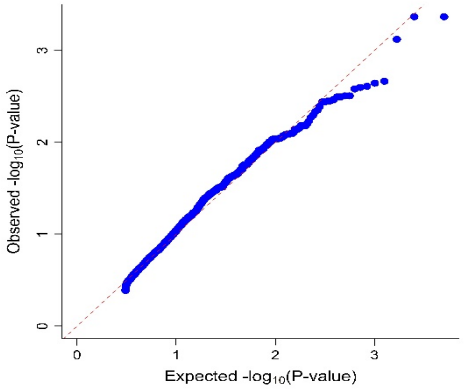


Fig 7.6b: QQ Plot ENV2-Awka Rain Yield

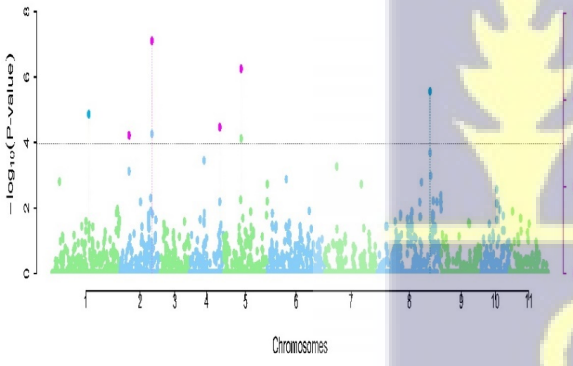


Fig. 7.7a: Manhattan Plot EVT 3-Uyo dry

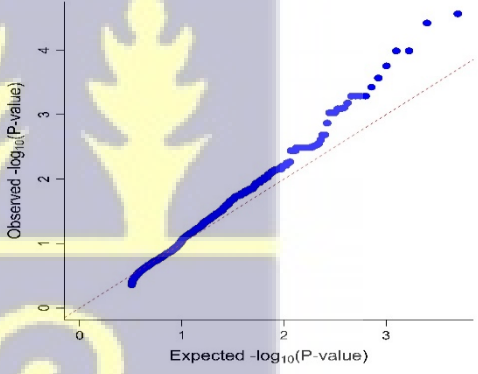


Fig.7.7b: QQ Plot EVT 3-Uyo dry Protein

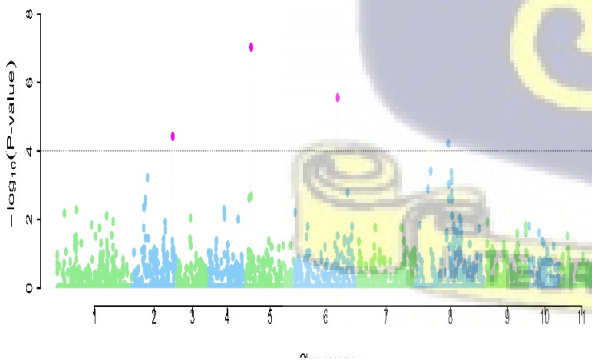


Fig. 7.8a: Manhattan Plot EVT 3-Uyo dry

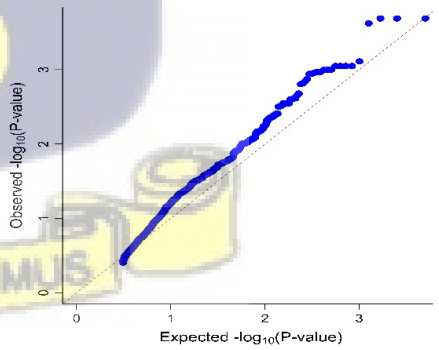


Fig. 7.8b: QQ Plot EVT 3-Uyo dry yield

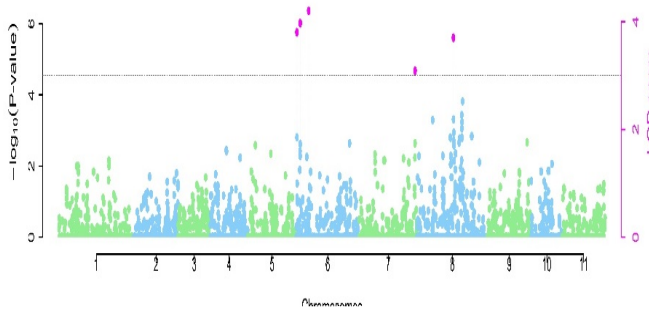


Fig. 7.9a: Manhattan Plot EVT 4-Uyo Rain Protein

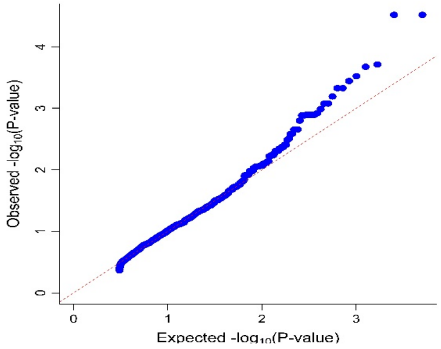


Fig. 7.9b: QQ Plot EVT 4-Uyo Rain Protein

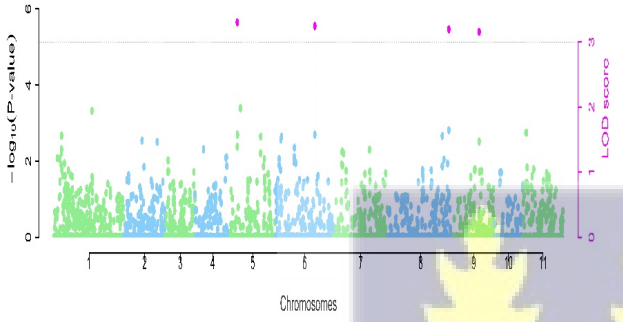


Fig. 7.10a: Manhattan Plot EVT 4-Uyo Rain Yield

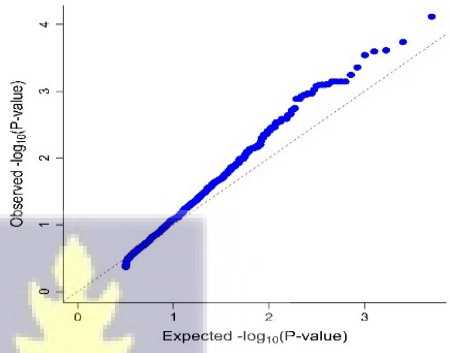


Fig. 7.10b: QQ Plot EVT 4-Uyo Rain Yield

Table 7.1 An overview of the major single nucleotide polymorphisms in a panel of 120 Vigna radiata genotypes that describe various genomic regions linked to the traits under study for four environments

| Traits | Methods | Markers | Chr | Pos/MP | Effect | Log10(P) | r2(%) | MAF | Var error |
|---|---|---|---|---|---|---|---|---|---|
| Protein | mrMLM | Chr1_2908989 | 1 | 2908989 | 0.0872 | 4.7571 | 12.7117 | 0.4831 | 0.0447 |
| | mrMLM | Chr3_7994659 | 3 | 7994659 | -0.0876 | 4.6905 | 12.4946 | 0.4153 | 0.0447 |
| | FASTmrMLM | Chr1_2908989 | 1 | 2908989 | 0.067 | 3.8261 | 7.8481 | 0.4833 | 0.0472 |
| | FASTmrMLM | Chr6_17778739 | 6 | 17778739 | 4.00E-04 | 3.7512 | 2.00E-04 | 0.2083 | 0.0472 |
| | FASTmrEMMA | Chr1_2908989 | 1 | 2908989 | 0.1334 | 3.9711 | 7.7659 | 0.4833 | 0.0473 |
| | FASTmrEMMA | Chr3_7994659 | 3 | 7994659 | -0.1329 | 4.0991 | 7.5096 | 0.4167 | 0.0473 |
| | pLARmEB | Chr1_12963638 | 1 | 12963638 | -0.0639 | 3.8012 | 4.9817 | 0.225 | 0.0474 |
| | mrMLM | Chr1_2908989 | 1 | 2908989 | 0.0872 | 4.7571 | 12.7117 | 0.4831 | 0.0447 |
| | | | | | | | | | |
| Yield | mrMLM | chr3_9213031 | 3 | 9213031 | -47.9039 | 4.217 | 8.1287 | 0.2034 | 16586.58 |
| | FASTmrMLM | chr3_9213031 | 3 | 213031 | -38.7016 | 3.9423 | 5.3056 | 0.2 | 15122.58 |
| | FASTmrMLM | chr4_19961226 | 4 | 19961226 | -59.4601 | 4.6601 | 7.5609 | 0.1083 | 15122.58 |
| | pLARmEB | chr3_9213031 | 3 | 9213031 | -38.8416 | 4.121 | 3.7834 | 0.2 | 14379.38 |
| | pLARmEB | chr4_19961226 | 4 | 19961226 | -51.93 | 3.8215 | 4.0829 | 0.1083 | 14379.38 |
| | ISIS EM-BLASSO | chr3_9213031 | 3 | 9213031 | -38.8416 | 4.121 | 5.3441 | 0.2 | 14379.38 |
| | ISIS EM-BLASSO | chr4_19961226 | 4 | 19961226 | -51.93 | 3.8215 | 5.7672 | 0.1083 | 14379.38 |
| | | | | | | | | | |
| Protein | pLARmEB | Chr2_809453 | 2 | 809453 | 8.00E-04 | 4.642 | 3.00E-04 | 0.15 | 0.0925 |
| | pLARmEB | Chr5_3185830 | 5 | 3185830 | 0.0591 | 6.2064 | 2.8415 | 0.3167 | 0.0925 |
| | pLARmEB | Chr5_25318711 | 5 | 25318711 | 0.0553 | 3.9737 | 2.8558 | 0.4583 | 0.0925 |
| | ISIS EM-BLASSO | chr8_30664570 | 8 | 30664570 | 0.1168 | 5.6587 | 10.4081 | 0.2833 | 0.0889 |
| | | | | | | | | | |
| Yield | mrMLM | Chr3_6148955 | 3 | 6148955 | -261.402 | 4.1667 | 12.2795 | 0.3136 | 422006.4 |
| | pLARmEB | chr3_6148955 | 3 | 6148955 | -212.091 | 4.6766 | 8.0837 | 0.3167 | 412243.6 |
| | ISIS EM-BLASSO | chr5_15655284 | 5 | 15655284 | 204.9571 | 4.8392 | 5.921 | 0.2167 | 363282.5 |
| | ISIS EM-BLASSO | chr6_29354314 | 6 | 29354314 | -258.754 | 3.8313 | 4.2475 | 0.0833 | 363282.5 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Protein | pLARmEB | chr6_569503 | 6 | 569503 | -0.075 | 4.5425 | 2.3367 | 0.125 | 0.0814 |
| | pLARmEB | chr6_3605082 | 6 | 3605082 | 2.90E-05 | 4.9597 | 5.28E-07 | 0.2083 | 0.0814 |
| | pLARmEB | chr8_31395733 | 8 | 31395733 | 0.0948 | 4.4355 | 6.6798 | 0.2667 | 0.0814 |
| | ISIS EM-BLASSO | chr6_1178767 | 6 | 1178767 | 1.00E-04 | 4.72 | 3.10E-06 | 0.05 | 0.097 |
| | ISIS EM-BLASSO | chr7_54291218 | 7 | 54291218 | 1.00E-04 | 3.7869 | 4.44E-06 | 0.0583 | 0.097 |
| | | | | | | | | | |
| Yield | mrMLM | chr2_21263131 | 2 | 21263131 | -256.509 | 6.0284 | 13.0781 | 0.0593 | 62749.04 |
| | mrMLM | chr2_2956559 | 2 | 2956559 | -152.126 | 3.9047 | 7.5366 | 0.0932 | 62749.04 |
| | mrMLM | chr4_19860552 | 4 | 19860552 | 93.3986 | 3.8563 | 7.3981 | 0.3729 | 62749.04 |
| | mrMLM | chr5_16189831 | 5 | 16189831 | -272.454 | 6.0323 | 12.7587 | 0.0508 | 62749.04 |
| | FASTmrMLM | chr2_2956559 | 2 | 2956559 | -118.432 | 3.8878 | 4.5678 | 0.1 | 72861.83 |
| | FASTmrMLM | chr2_21263131 | 2 | 21263131 | -204.665 | 4.9909 | 8.3259 | 0.0583 | 72861.83 |
| | FASTmrMLM | chr5_16189831 | 5 | 16189831 | -190.152 | 4.0422 | 6.2147 | 0.05 | 72861.83 |
| | pLARmEB | chr1_12467544 | 1 | 12467544 | 65.321 | 4.4098 | 0.5672 | 0.2167 | 54604.7 |
| | pLARmEB | chr2_2956559 | 2 | 2956559 | -99.3282 | 3.8104 | 0.6955 | 0.1 | 54604.7 |
| | pLARmEB | chr2_21263131 | 2 | 21263131 | -206.487 | 6.6036 | 1.8345 | 0.0583 | 54604.7 |
| | pLARmEB | chr4_19860552 | 4 | 19860552 | 58.7282 | 4.4139 | 0.6332 | 0.375 | 54604.7 |
| | pLARmEB | Chr5_16189831 | 5 | 16189831 | -204.904 | 5.921 | 1.5621 | 0.05 | 54604.7 |
| | pLARmEB | chr8_42327370 | 8 | 42327370 | -147.15 | 4.9615 | 1.0553 | 0.0667 | 54604.7 |
| | ISIS EM-BLASSO | chr2_2956559 | 2 | 2956559 | -123.014 | 4.3715 | 4.9281 | 0.1 | 66333.07 |
| | ISIS EM-BLASSO | chr2_21263131 | 2 | 21263131 | -223.521 | 6.3294 | 9.9307 | 0.0583 | 66333.07 |
| | ISIS EM-BLASSO | chr4_19860552 | 4 | 19860552 | 63.1468 | 4.0963 | 3.3817 | 0.375 | 66333.07 |
| | ISIS EM-BLASSO | chr5_16189831 | 5 | 16189831 | -208.156 | 5.093 | 7.4473 | 0.05 | 66333.07 |
| | | | | | | | | | |
| Protein | pLARmEB | chr2_24615287 | 2 | 24615287 | 1.39E-05 | 4.0298 | 1.76E-07 | 0.3917 | 0.0868 |
| | pLARmEB | chr5_3185830 | 5 | 3185830 | 0.1124 | 6.0667 | 10.3769 | 0.3167 | 0.0868 |
| | pLARmEB | chr6_31253548 | 6 | 31253548 | 0.0704 | 4.9175 | 4.0721 | 0.3167 | 0.0868 |
| | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Yield | mrMLM | chr5_5871534 | 5 | 5871534 | 502.2993 | 4.0129 | 7.9464 | 0.0763 | 666349.4 |
| | mrMLM | chr6_30896900 | 6 | 30896900 | -448.201 | 3.9529 | 9.5709 | 0.1356 | 666349.4 |
| | mrMLM | chr8_44276921 | 8 | 44276921 | -549.825 | 3.8986 | 6.8466 | 0.0593 | 666349.4 |
| | mrMLM | chr9_10783673 | 9 | 10783673 | -432.834 | 3.8578 | 6.9519 | 0.1017 | 666349.4 |

**TABLE 7.2 Gene identification for the significant SNPs for studied traits.**

| Environment | Traits | Methods | Markers | Chr | Pos/MP | Function |
|---|---|---|---|---|---|---|
| | | mrMLM | Chr1_2908989 | 1 | 2908989 | EC_6.2 ligase forming carbon-sulfur |
| | | mrMLM | Chr3_7994659 | 3 | 7994659 | Resection MRE11-RAD50-NBS1 (MRN) |
| | | FASTmrMLM | Chr1_2908989 | 1 | 2908989 | EC_6.2 ligase forming carbon-sulfur |
| | | FASTmrMLM | Chr6_17778739 | 6 | 17778739 | AS2/LOB-type transcription factor |
| | | FASTmrEMMA | Chr1_2908989 | 1 | 2908989 | EC_6.2 ligase forming carbon-sulfur |
| | | FASTmrEMMA | Chr3_7994659 | 3 | 7994659 | Resection MRE11-RAD50-NBS1 (MRN) |
| | | pLARmEB | Chr1_12963638 | 1 | 12963638 | LRK91_ARATH (Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK)) |
| Awka Dry | Protein | mrMLM | Chr1_2908989 | 1 | 2908989 | EC_6.2 ligase forming carbon-sulfur |
| | Yield | mrMLM | chr3_9213031 | 3 | 9213031 | EC_2.1 transferase transferring one-carbon |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | FASTmrMLM | chr3_9213031 | 3 | 213031 | EC_2.1 transferase transferring one-carbon |
| | | FASTmrMLM | chr4_19961226 | 4 | 19961226 | Unclassified protein |
| | | pLARmEB | chr3_9213031 | 3 | 9213031 | EC_2.1 transferase transferring one-carbon |
| | | pLARmEB | chr4_19961226 | 4 | 19961226 | Uncharacterized protein |
| | | ISIS EM-BLASSO | chr3_9213031 | 3 | 9213031 | EC_2.1 transferase transferring one-carbon |
| | | ISIS EM-BLASSO | chr4_19961226 | 4 | 19961226 | Uncharacterized protein |
| | | | | | | |
| Awka Rain | Protein | pLARmEB | Chr2_809453 | 2 | 809453 | SRS1(SHI related sequence 1) SRS-type transcription facto |
| | | pLARmEB | Chr5_3185830 | 5 | 3185830 | F-Box Protein (FB49_ARATH (XP_014517464.1)) |
| | | pLARmEB | Chr5_25318711 | 5 | 25318711 | Transcription Elongation Factor B Polypeptide (XP_014522546.1) |
| | | ISIS EM-BLASSO | chr8_30664570 | 8 | 30664570 | RL4/uL4 ribosomal protein (XP_014491920.1) |
| | | | | | | |
| | Yield | mrMLM | Chr3_6148955 | 3 | 6148955 | EGC_CITJA; XP_014504277.1; PNP precursor polypeptide |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | pLARmEB | chr3_6148955 | 3 | 6148955 | EGC_CITJA; XP_014504277.1; PNP precursor polypeptide |
| | | ISIS EM-BLASSO | chr5_15655284 | 5 | 15655284 | Embryogenesis abundant protein; XP_014517278.1 |
| | | ISIS EM-BLASSO | chr6_29354314 | 6 | 29354314 | Microtubule-associated protein crucial for spindle assembly during cell division spF4I2H7\|TPX2_ARATH; XP_014493011.1 |
| | | | | | | |
| Uyo Dry | Protein | pLARmEB | chr6_569503 | 6 | 569503 | C2H2 Zinc Finger Transcription factor |
| | | pLARmEB | chr6_3605082 | 6 | 3605082 | Uncharacterized proteinLOC106755679 from Vigna radiata var. radiata (mung bean). |
| | | pLARmEB | chr8_31395733 | 8 | 31395733 | Uncharacterized protein |
| | | ISIS EM-BLASSO | chr6_1178767 | 6 | 1178767 | EC_3.4 hydrolase acting on peptide bond |
| | | ISIS EM-BLASSO | chr7_54291218 | 7 | 54291218 | Uncharacterized protein |
| | | | | | | |
| | Yield | mrMLM | chr2_21263131 | 2 | 21263131 | Uncharacterised protein |
| | | mrMLM | chr2_2956559 | 2 | 2956559 | BKI1 (Brassinosteroid Kinase Inhibitor 1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | mrMLM | chr4_19860552 | 4 | 19860552 | Uncharacterised protein |
| | | mrMLM | chr5_16189831 | 5 | 16189831 | Fasciclin-Type Arabinogalactan Protein (FLA1) |
| | | FASTmrMLM | chr2_2956559 | 2 | 2956559 | BKI1 (Brassinosteroid Kinase Inhibitor 1) |
| | | FASTmrMLM | chr2_21263131 | 2 | 21263131 | Uncharacterised protein |
| | | FASTmrMLM | chr5_16189831 | 5 | 16189831 | Fasciclin-Type Arabinogalactan Protein (FLA1) |
| | | pLARmEB | chr1_12467544 | 1 | 12467544 | uncharacterized protein |
| | | pLARmEB | chr2_2956559 | 2 | 2956559 | BKI1 (Brassinosteroid Kinase Inhibitor 1) |
| | | pLARmEB | chr2_21263131 | 2 | 21263131 | uncharacterized protein |
| | | pLARmEB | chr4_19860552 | 4 | 19860552 | uncharacterized protein |
| | | pLARmEB | Chr5_16189831 | 5 | 16189831 | Fasciclin-Type Arabinogalactan Protein (FLA1) |
| | | pLARmEB | chr8_42327370 | 8 | 42327370 | uncharacterized protein |
| | | ISIS EM-BLASSO | chr2_2956559 | 2 | 2956559 | BKI1 (Brassinosteroid Kinase Inhibitor 1) |
| | | ISIS EM-BLASSO | chr2_21263131 | 2 | 21263131 | uncharacterized protein |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | ISIS EM-BLASSO | chr4_19860552 | 4 | 19860552 | Uncharacterised protein |
| | | ISIS EM-BLASSO | chr5_16189831 | 5 | 16189831 | Fasciclin-Type Arabinogalactan Protein (FLA1) |
| | | | | | | |
| Uyo Rain | Protein | pLARmEB | chr2_24615287 | 2 | 24615287 | Uncharacterised protein |
| | | pLARmEB | chr5_3185830 | 5 | 3185830 | FB49_ARATH (XP_014517464.1) regulates protein degradation through the ubiquitin-proteasome system (UPS). |
| | | pLARmEB | chr6_31253548 | 6 | 31253548 | putative zinc finger protein CONSTANS-LIKE 11 |
| | | | | | | |
| | Yield | mrMLM | chr5_5871534 | 5 | 5871534 | ATPase family; sp\|P54774\|CDC48_SOYBN; XP_014518955.1; |
| | | mrMLM | chr6_30896900 | 6 | 30896900 | THYLAKOID FORMATION1; regulatory protein *(THF1) of thylakoid |
| | | mrMLM | chr8_44276921 | 8 | 44276921 | Transcription elongation factor B polypeptide |
| | | mrMLM | chr9_10783673 | 9 | 10783673 | Mitochondrialcarrier (TC2.A.29)family;sp\|O65023\|EAAC_ARATH;XP_014515983.1;solute transporter |

### 7.3.5 Discussion

Genome-wide association studies (GWAS) were conducted across four environments (Awka Dry, Awka Rain, Uyo Dry, and Uyo Rain) to identify genomic regions associated with protein content and yield in mung bean (Vigna radiata). Several candidate genes were identified that may play a role in regulating these complex traits. The identification of these regions in varying environments is important for developing Mungbean varieties that can thrive in differing environments.

#### 7.3.6.1 Protein Content

In the Awka Dry environment, several markers were significantly associated with protein content. A marker on chromosome 1 (Chr1_2908989) was consistently linked to EC 6.2 ligase forming carbon-sulfur. Ligases play crucial roles in cellular metabolism, including the synthesis of amino acids, which are the building blocks of proteins (Lodish et al., 2000). Specifically, carbon-sulfur ligases are important for methionine biosynthesis, a key amino acid in plant metabolism. Additionally, a marker on chromosome 3 (Chr3_7994659) was associated with Resection MRE11-RAD50-NBS1 (MRN), a complex involved in DNA repair and recombination. Although primarily known for its role in DNA maintenance, the MRN complex has also been implicated in regulating gene expression (Haber, 1998), suggesting a potential indirect effect on protein synthesis. Furthermore, a marker on chromosome 6 (Chr6_17778739) was associated with AS2/LOB-type transcription factor. AS2/LOB-domain proteins are plant-specific transcription factors involved in various developmental processes (Iwakawa et al., 2002). Homologs of LOB-domain proteins in legumes are known to regulate nodule development, a process vital for nitrogen fixation and, consequently, protein synthesis.

In Awka Rain, the SRS1 (SHI-related sequence 1) SRS-type transcription factor on chromosome 2 (Chr2_809453) was associated with protein content. SRS1 transcription factors

are known to be involved in plant development and hormone signalling (Rate et al., 1999). Given the role of the SRS family in regulating developmental processes, it is plausible that variations in this gene impact protein allocation in mung bean. A marker on chromosome 5 (Chr5_3185830) was associated with F-Box Protein (FB49_ARATH (XP_014517464.1)). F-box proteins are components of the SCF (Skp1-Cullin-F-box) ubiquitin ligase complex, which regulates protein degradation (Kepinski & Leyser, 2005). It is likely that this F-box protein influences the turnover of proteins within the mung bean seed, thereby affecting overall protein content. The ubiquitination pathway is essential for the efficient utilization of resources in plants (Vierstra, 2009).

In Uyo Dry, several uncharacterized proteins were associated with protein content, highlighting the novelty and potential for discovery in this region. Given the close evolutionary relationship between Arabidopsis thaliana and Vigna radiata, it is plausible that these uncharacterized proteins could be novel targets for improving mung bean protein quality. The finding highlights the importance of exploring the role of the C2H2 Zinc Finger Transcription factor* found on chromosome 6. Zinc finger proteins are key transcription factors involved in the regulation of gene expression in many plant processes (Klug, 2010). A specific uncharacterized protein LOC106755679 from Vigna radiata var. radiata was also identified as a contributing factor. A hydrolase acting on a peptide bond was also significantly linked to protein content (Rawlings et al., 2018).

Uyo Rain shared the same F-Box Protein (FB49_ARATH (XP_014517464.1) with Awka Rain, highlighting a consistent role for this protein under different environmental conditions. However, in Uyo Rain environment, putative zinc finger protein CONSTANS-LIKE 11 (chr6_31253548) was also a significant marker. CONSTANS-LIKE proteins are transcription factors involved in the photoperiod pathway and flowering time control (Valverde, 2011),

indicating a potential link between flowering and protein content. Mungbean is highly sensitive to photoperiod, therefore this is a valid consideration to the crop.

#### 7.3.6.2 Yield

For Awka Dry, EC 2.1 transferase transferring one-carbon was significantly linked to yield, playing an important role in one carbon metabolism (Appling, 1991). One-carbon metabolism is essential for synthesizing nucleotides, amino acids, and other essential metabolites, directly affecting plant growth and yield. Additionally, an uncharacterized protein was also significant.

In the Awka Rain environment, a marker on chromosome 3 (Chr3_6148955) was associated with yield and annotated as EGC_CITJA;XP_014504277.1;PNP precursor polypeptide. Polynucleotide phosphorylase (PNP) is involved in RNA degradation and processing, which can affect gene expression and plant development (Yehudai-Resheff et al., 2001). Given that PNP is essential for RNA degradation and processing, variations in this gene may impact the stability and translation efficiency of key mRNAs involved in mung bean yield.

In Uyo Dry, BKI1 (Brassinosteroid Kinase Inhibitor 1) and Fasciclin-Type Arabinogalactan Protein (FLA1) was significantly linked to yield. BKI1 negatively regulates Brassinosteroid signaling, which plays a crucial role in plant growth and development. Because Brassinosteroid are essential for plant growth and development, the variation may also affect yield. Fasciclin-type arabinogalactan proteins (FLAs) are cell wall proteins involved in cell adhesion and expansion, which can affect plant architecture and yield. It is plausible that FLAs influence cell wall properties in mung bean, thereby affecting overall plant architecture and yield. Cell wall structure is essential for plant strength and stability; therefore, this finding is also valid.

In Uyo Rain, several markers were linked to yield, including AAA ATPasefamily; sp|P54774|CDC48_SOYBN;XP_014518955.1;;THYLAKOID FORMATION1;regulatory

protein \(THF1) of thylakoid, Transcription elongation factor B polypeptide and mitochondrial carrier (TC 2.A.29) family. AAA ATPases are involved in various cellular processes, including protein degradation and DNA repair (Neuwald et al., 1999), and a mutation in this protein would affect yield. THF1 is essential for thylakoid membrane biogenesis, which is critical for photosynthesis (Peng et al., 2006). Since mung bean yield is highly dependent on photosynthetic efficiency, this is a key gene for influencing yield. Transcription elongation factor B polypeptide regulates the rate of transcription elongation, influencing gene expression levels (Orphanides et al., 1998). A study found that this polypeptide in Arabidopsis is linked to plant responses to temperature stress (Van Dijk et al., 2015).

### 7.4 Conclusion

This study successfully identified 16 significant marker-trait associations (MTAs) for yield and 10 MTAs for protein content in mungbean using DArTseq-based genome-wide association studies (GWAS). These MTAs were distributed across multiple chromosomes, with protein content markers found on chromosomes 1, 3, 5, and 6, while yield-associated markers were identified on chromosomes 2, 3, 4, 5, 6, 8, and 9. The identification of these loci provides a valuable genomic resource for marker-assisted selection (MAS), facilitating the breeding of high-yielding and protein-rich mungbean varieties for enhanced nutritional value.



## CHAPTER EIGHT

## 8.0 General Conclusion

This study provides a comprehensive assessment of the phenotypic diversity, genetic variation, and environmental adaptability of 120 mungbean genotypes across four agro-ecological environments in Nigeria (Awka and Uyo, during both dry and rainy seasons). By integrating phenotypic characterization, genomic sequencing (DArTseq), stability analysis, and genome-wide association studies (GWAS), this research successfully identified superior mungbean genotypes for yield stability and protein content enhancement. Genetic diversity and SNP identification uncovered a total of 5,037 high-quality SNPs were identified, distributed across 11 chromosomes, Chromosome 1 had the highest SNP density (689 SNPs, 13.68%). Stability analysis using AMMI and GGE biplot models identified genotypes 130, 105, and 20 as the most stable for yield, while genotype 130 was optimal for protein content stability. Uyo (rainy season) was identified as the best environment for optimizing both yield and protein content. A total of 16 significant MTAs for yield were identified, located on chromosomes 5, 6, 8, and 9. A total of 10 significant MTAs for protein content were found, mapped to chromosomes 1, 2, 3, 5, and 6. Functional gene annotation suggested that some of these loci encode enzymes involved in carbon-sulfur metabolism, transcription factors, and nutrient transporters, which influence grain composition. These findings emphasize the importance of molecular breeding, marker-assisted selection (MAS), and genotype-by-environment interaction studies in improving mungbean productivity. The results provide valuable genomic resources for future breeding efforts aimed at enhancing yield, protein content, and environmental resilience in mungbean



## 8.1 Recommendations

Based on the findings of this study, the following recommendations are proposed:

8.2. Breeding and Genetic Improvement

- Marker-assisted selection (MAS) should be prioritized to develop superior mungbean varieties with enhanced yield and protein content.
- Further validation of identified MTAs through functional genomics is needed to confirm the candidate genes responsible for yield and protein accumulation.
- Incorporation of genotypes 130, 105, and 20 in breeding programs is recommended due to their yield stability and adaptability.

8.3. Agronomic and Environmental Management

- Multi-location field trials should be expanded to additional agro-ecological zones in Nigeria to capture broader environmental interactions.
- Site-specific recommendations should be developed, particularly focusing on optimizing irrigation, soil fertility management, and planting season selection.
- Uyo (rainy season) should be promoted as a key location for large-scale mungbean production due to its optimal conditions for both yield and protein accumulation.

8.4. Future Research Directions

- Genome editing techniques (CRISPR-Cas9) and transcriptomics should be explored to validate gene functions influencing protein accumulation and yield stability.
- Studies on biotic and abiotic stress tolerance should be integrated with GWAS to develop mungbean varieties resilient to drought, pests, and diseases.
- Nutritional and food processing studies should be conducted to optimize mungbean's use in food formulations, addressing protein malnutrition in Nigeria.

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**Appendix I**

**Tables 2: List of 299 Genotypes of mungbean used in this Study with their sources**

| Serial no. | MMC no. | VI no. | Source |
|---|---|---|---|
| 1 | 1 | VI000020 AY | Thailand |
| 2 | 2 | VI000099 AG | India |
| 3 | 3 | VI000105 BG | India |
| 4 | 4 | VI000164 BG | Afghanistan |
| 5 | 5 | VI000170 B-BR | Afghanistan |
| 6 | 6 | VI000175 BY | India |
| 7 | 7 | VI000188 A-BLM | Pakistan |
| 8 | 8 | VI000203 B-BR | Afghanistan |
| 9 | 9 | VI000212 A-BLM | United States of America |
| 10 | 10 | VI000232 AG | Iran |
| 11 | 11 | VI000238 AG | Afghanistan |
| 12 | 12 | VI000253 AG | India |
| 13 | 13 | VI000316 AG | Pakistan |
| 14 | 14 | VI000317 BG | Pakistan |
| 15 | 15 | VI000319 AG | Pakistan |
| 16 | 16 | VI000380 AG | Philippines |
| 17 | 17 | VI000461 BG | Thailand |
| 18 | 18 | VI000470 AG | Pakistan |
| 19 | 19 | VI000532 BG | India |
| 20 | 20 | VI000537 BG | India |
| 21 | 21 | VI000542 BY | India |
| 22 | 22 | VI000551 AG | India |
| 23 | 23 | VI000554 AG | India |
| 24 | 24 | VI000559 AG | India |
| 25 | 25 | VI000578 AG | India |
| 26 | 26 | VI000589 B-BR | India |
| 27 | 27 | VI000616 BG | Brazil |
| 28 | 28 | VI000618 AG | India |
| 29 | 29 | VI000625 B-BR | India |
| 30 | 30 | VI000680 AG | United States of America |
| 31 | 31 | VI000723 AG | Iran |
| 32 | 32 | VI000732 AG | India |
| 33 | 33 | VI000735 BG | India |
| 34 | 34 | VI000736 AG | India |
| 35 | 35 | VI000749 AG | India |

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|---|---|---|---|
| 36 | 36 | VI000764 AG | India |
| 37 | 37 | VI000766 BG | India |
| 38 | 38 | VI000805 BG | India |
| 39 | 39 | VI000815 BG | India |
| 40 | 40 | VI000818 BG | India |
| 41 | 41 | VI000852 AG | India |
| 42 | 42 | VI000938 AG | India |
| 43 | 43 | VI000942 AG | India |
| 44 | 44 | VI000953 AG | India |
| 45 | 45 | VI000981 BG | Philippines |
| 46 | 46 | VI001023 BG | India |
| 47 | 47 | VI001066 BG | Australia |
| 48 | 48 | VI001096 AG | Australia |
| 49 | 49 | VI001124 AG | Australia |
| 50 | 50 | VI001126 BG | Australia |
| 51 | 51 | VI001162 AG | Australia |
| 52 | 52 | VI001191 BG | Philippines |
| 53 | 53 | VI001211 AG | Philippines |
| 54 | 54 | VI001221 AG | Philippines |
| 55 | 55 | VI001244 AG | Philippines |
| 56 | 56 | VI001268 BG | India |
| 57 | 57 | VI001282 AG | India |
| 58 | 58 | VI001284 AG | India |
| 59 | 59 | VI001339 AG | Philippines |
| 60 | 60 | VI001385 AG | India |
| 61 | 61 | VI001400 AG | India |
| 62 | 62 | VI001403 BR | India |
| 63 | 63 | VI001406 BG | Pakistan |
| 64 | 64 | VI001408 BG | India |
| 65 | 65 | VI001411 AG | India |
| 66 | 66 | VI001412 AG | India |
| 67 | 67 | VI001419 BG | India |
| 68 | 68 | VI001435 AG | United States of America |
| 69 | 69 | VI001448 A-BLM | India |
| 70 | 70 | VI001471 AG | India |
| 71 | 71 | VI001482 BG | India |
| 72 | 72 | VI001490 AG | Iran |
| 73 | 73 | VI001509 AG | Pakistan |
| 74 | 74 | VI001514 AG | India |

| | | | |
|---|---|---|---|
| 75 | 75 | VI001520 A-BLM | India |
| 76 | 76 | VI001533 BG | India |
| 77 | 77 | VI001535 BG | India |
| 78 | 78 | VI001539 AG | India |
| 79 | 79 | VI001548 AG | India |
| 80 | 80 | VI001556 BG | India |
| 81 | 81 | VI001557 BG | United States of America |
| 82 | 82 | VI001562 AG | India |
| 83 | 83 | VI001576 BG | India |
| 84 | 84 | VI001579 BG | India |
| 85 | 85 | VI001605 BG | India |
| 86 | 86 | VI001612 AG | Unknown |
| 87 | 87 | VI001628 AG | India |
| 88 | 88 | VI001651 BG | India |
| 89 | 89 | VI001652 BG | India |
| 90 | 90 | VI001654 BG | India |
| 91 | 91 | VI001678 BG | India |
| 92 | 92 | VI001692 AG | India |
| 93 | 93 | VI001698 BG | India |
| 94 | 94 | VI001728 AG | India |
| 95 | 95 | VI001733 BG | India |
| 96 | 96 | VI001743 BG | India |
| 97 | 97 | VI001756 BG | India |
| 98 | 98 | VI001762 A-GM | India |
| 99 | 99 | VI001806 AG | Pakistan |
| 100 | 100 | VI001806 BG | **Pakistan** |
| 101 | 101 | VI001820 BG | France |
| 102 | 102 | VI001859 BG | Thailand |
| 103 | 103 | VI001974 BG | Korea, Republic of |
| 104 | 104 | VI001993 BG | Korea, Republic of |
| 105 | 105 | VI002009 BG | India |
| 106 | 106 | VI002012 BG | India |
| 107 | 107 | VI002051 BG | India |
| 108 | 108 | VI002063 BG | United States of America |
| 109 | 109 | VI002173 AG | India |
| 110 | 110 | VI002173 BG | **India** |
| 111 | 111 | VI002176 AG | India |
| 112 | 112 | VI002176 BG | **India** |

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|---|---|---|---|
| 113 | 113 | VI002190 BG | India |
| 114 | 114 | VI002195 AG | Thailand |
| 115 | 115 | VI002197 BG | Korea, Republic of |
| 116 | 116 | VI002206 AG | Philippines |
| 117 | 117 | VI002239 AG | Afghanistan |
| 118 | 119 | VI002284 BG | Afghanistan |
| 119 | 120 | VI002402 BG | Thailand |
| 120 | 121 | VI002432 AG | Thailand |
| 121 | 122 | VI002437 BG | Korea, Republic of |
| 122 | 123 | VI002456 AG | Korea, Republic of |
| 123 | 124 | VI002469 AG | Philippines |
| 124 | 125 | VI002487 AG | Pakistan |
| 125 | 126 | VI002523 AG | Thailand |
| 126 | 128 | VI002532 AG | India |
| 127 | 129 | VI002537 AG | Turkey |
| 128 | 130 | VI002569 BG | Nigeria |
| 129 | 131 | VI002587 AG | Australia |
| 130 | 132 | VI002611 AG | Thailand |
| 131 | 133 | VI002646 AG | Thailand |
| 132 | 134 | VI002647 AG | Thailand |
| 133 | 135 | VI002672 AG | Thailand |
| 134 | 136 | VI002739 AG | Iran |
| 135 | 137 | VI002802 A-BR | Iran |
| 136 | 138 | VI002859 BG | Iran |
| 137 | 139 | VI002860 AG | Iran |
| 138 | 140 | VI002872 BG | Iran |
| 139 | 141 | VI002877 BG | Iran |
| 140 | 142 | VI002894 B-BR | Iran |
| 141 | 143 | VI002926 AG | India |
| 142 | 144 | VI002934 AG | India |
| 143 | 145 | VI002986 AG | India |
| 144 | 146 | VI002993 BG | India |
| 145 | 147 | VI002999 AG | India |
| 146 | 148 | VI003019 A-BLM | Unknown |
| 147 | 149 | VI003019 BG | **Unknown** |
| 148 | 150 | VI003034 BG | India |
| 149 | 151 | VI003035 AG | India |
| 150 | 152 | VI003057 BG | India |
| 151 | 153 | VI003062 BG | India |

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|---|---|---|---|
| 152 | 154 | VI003068 A-BR | India |
| 153 | 155 | VI003070 AG | India |
| 154 | 156 | VI003083 BG | India |
| 155 | 157 | VI003114 AG | India |
| 156 | 158 | VI003135 B-BL | India |
| 157 | 159 | VI003159 AG | India |
| 158 | 160 | VI003172 BG | India |
| 159 | 161 | VI003181 B-GM | India |
| 160 | 162 | VI003183 AG | India |
| 161 | 163 | VI003187 BG | India |
| 162 | 164 | VI003212 B-BLM | India |
| 163 | 165 | VI003220 AG | India |
| 164 | 166 | VI003232 AG | India |
| 165 | 167 | VI003235 AG | India |
| 166 | 168 | VI003242 AG | India |
| 167 | 169 | VI003251 A-BL | India |
| 168 | 170 | VI003251 A-BLM | **India** |
| 169 | 171 | VI003252 BG | India |
| 170 | 172 | VI003255 AG | India |
| 171 | 173 | VI003276 BG | India |
| 172 | 174 | VI003329 AG | India |
| 173 | 175 | VI003332 AG | India |
| 174 | 176 | VI003337 BG | India |
| 175 | 177 | VI003364 AG | India |
| 176 | 178 | VI003379 BG | India |
| 177 | 179 | VI003382 BG | India |
| 178 | 180 | VI003407 AG | India |
| 179 | 181 | VI003413 BG | India |
| 180 | 182 | VI003440 AG | India |
| 181 | 183 | VI003455 AG | India |
| 182 | 184 | VI003456 AG | Unknown |
| 183 | 185 | VI003465 BG | India |
| 184 | 186 | VI003470 BG | India |
| 185 | 187 | VI003480 BG | India |
| 186 | 188 | VI003490 AG | India |
| 187 | 189 | VI003493 BG | India |
| 188 | 190 | VI003514 BG | India |

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|---|---|---|---|
| 189 | 191 | VI003517 BG | India |
| 190 | 192 | VI003534 AG | India |
| 191 | 193 | VI003534 BG | India |
| 192 | 194 | VI003548 AG | India |
| 193 | 195 | VI003554 AG | India |
| 194 | 196 | VI003560 BG | India |
| 195 | 197 | VI003563 A-BR | India |
| 196 | 198 | VI003577 AG | India |
| 197 | 199 | VI003602 AG | India |
| 198 | 200 | VI003642 AG | India |
| 199 | 201 | VI003648 BG | India |
| 200 | 202 | VI003658 BG | India |
| 201 | 203 | VI003664 AG | India |
| 202 | 204 | VI003678 BG | India |
| 203 | 205 | VI003685 AG | India |
| 204 | 206 | VI003699 BG | India |
| 205 | 207 | VI003720 BG | India |
| 206 | 208 | VI003725 BG | India |
| 207 | 209 | VI003733 BG | India |
| 208 | 210 | VI003734 B-BR | India |
| 209 | 211 | VI003734 B-DG | **India** |
| 210 | 212 | VI003744 AG | India |
| 211 | 213 | VI003755 BG | India |
| 212 | 214 | VI003760 BG | India |
| 213 | 215 | VI003785 BG | India |
| 214 | 216 | VI003795 AG | India |
| 215 | 217 | VI003801 BG | India |
| 216 | 218 | VI003882 A-BLM | Afghanistan |
| 217 | 219 | VI003886 B-BR | India |
| 218 | 220 | VI003886 BY | India |
| 219 | 221 | VI003893 AG | India |
| 220 | 222 | VI003894 B-BLM | India |
| 221 | 223 | VI003907 AG | Iraq |
| 222 | 224 | VI003914 AG | India |
| 223 | 225 | VI003925 B-BLM | India |
| 224 | 226 | VI003927 AG | India |

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| 225 | 227 | VI003929 A-BL | India |
| 226 | 228 | VI003942 AG | Afghanistan |
| 227 | 229 | VI003944 B-BR | Afghanistan |
| 228 | 230 | VI003947 B-BR | India |
| 229 | 231 | VI003948 B-BR | India |
| 230 | 232 | VI003951 AG | India |
| 231 | 233 | VI003954 BG | India |
| 232 | 234 | VI003957 AG | India |
| 233 | 235 | VI003958 B-BLM | India |
| 234 | 236 | VI003959 BG | India |
| 235 | 237 | VI004006 A-GM | India |
| 236 | 238 | VI004010 AG | India |
| 237 | 239 | VI004024 AG | Australia |
| 238 | 240 | VI004044 BG | India |
| 239 | 241 | VI004045 A-DGM | India |
| 240 | 242 | VI004048 A-DGM | India |
| 241 | 243 | VI004069 BG | India |
| 242 | 244 | VI004096 AG | India |
| 243 | 245 | VI004096 BG | India |
| 244 | 246 | VI004129 A-BLM | Unknown |
| 245 | 247 | VI004138 BG | India |
| 246 | 248 | VI004145 B-BLM | Afghanistan |
| 247 | 249 | VI004184 AG | Netherlands |
| 248 | 250 | VI004243 B-BR | Turkey |
| 249 | 251 | VI004244 B-BR | India |
| 250 | 252 | VI004297 AG | Afghanistan |
| 251 | 253 | VI004302 AG | Afghanistan |
| 252 | 254 | VI004307 AG | Afghanistan |
| 253 | 255 | VI004312 AG | India |
| 254 | 256 | VI004347 B-BLM | India |
| 255 | 257 | VI004351 AG | India |
| 256 | 258 | VI004423 AG | Iran |

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| 257 | 259 | VI004432 B-BR | Iran |
| 258 | 260 | VI004480 AG | Iran |
| 259 | 261 | VI004639 AG | Iran |
| 260 | 262 | VI004666 AG | Iran |
| 261 | 263 | VI004691 AG | Iran |
| 262 | 264 | VI004694 BG | Iran |
| 263 | 265 | VI004710 AG | Iran |
| 264 | 266 | VI004734 AG | Iran |
| 265 | 267 | VI004743 AG | India |
| 266 | 268 | VI004789 BG | India |
| 267 | 269 | VI004810 BG | India |
| 268 | 270 | VI004811 BG | India |
| 269 | 271 | VI004822 BG | India |
| 270 | 272 | VI004838 AG | India |
| 271 | 273 | VI004842 AG | India |
| 272 | 274 | VI004853 BG | India |
| 273 | 275 | VI004871 BG | India |
| 274 | 276 | VI004877 AG | India |
| 275 | 277 | VI004915 BG | India |
| 276 | 278 | VI004931 AG | Pakistan |
| 277 | 279 | VI004933 AG | Pakistan |
| 278 | 280 | VI004934 AG | Pakistan |
| 279 | 281 | VI004937 AG | Pakistan |
| 280 | 282 | VI004942 BG | Pakistan |
| 281 | 283 | VI004954 BG | Pakistan |
| 282 | 284 | VI004956 AG | Pakistan |
| 283 | 285 | VI004957 AG | Pakistan |
| 284 | 286 | VI004958 BG | Pakistan |
| 285 | 287 | VI004965 BG | Pakistan |
| 286 | 288 | VI004968 AG | Pakistan |
| 287 | 289 | VI004969 AG | Pakistan |
| 288 | 290 | VI004973 B-BLM | India |
| 289 | 291 | VI005022 BG | India |
| 290 | 293 | VI005030 BY | Mexico |
| 291 | 294 | VI005041 AG | Unknown |
| 292 | 295 | VI005066 A-GM | India |
| 293 | 296 | VI014178 BG | Kenya |
| 294 | 297 | N/A | Nigeria |
| 295 | 298 | N/A | Nigeria |

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| 296 | 299 | N/A | Nigeria |
| 297 | 300 | N/A | Nigeria |
| 298 | 301 | N/A | Nigeria |
| 299 | 301 | N/A | Nigeria |